PROTEIN NUTRITION IN THE JUVENILE AUSTRALIAN SHORT-FINNED EEL (*Anguilla australis australis* Richardson)

by

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DECLARATION AND AUTHORITY OF ACCESS

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Kenan Engin

ABSTRACT

Little is known about the nutrition of the Australian short-finned eel, *Anguilla.australis.australis* (Richardson) although it is considered as a prime candidate for inland aquaculture in Australia. This study provides information about the protein metabolism of the juvenile Australian short-finned eel. The efficiency of partitioning dietary protein into growth is closely related to the amount of non-protein energy yielding substrates and the quality of protein (the availability and balance of amino acids) sources provided in the diet. The measurement of nitrogenous excretion can also give an insight into the nitrogen balance of fish and partly define the success of a particular nutritional regimen. Therefore, this study aimed at measuring the growth, growth efficiency and nitrogenous excretion of the juvenile Australian short-finned eel fed over a range of dietary protein:energy ratios and with selected Australian plant and animal proteins.

Since the maximum protein growth will occur over a narrow range of dietary protein:energy ratios, the effects of increasing dietary crude protein contents at two different energy levels on the growth and growth efficiency were measured. A 10 % increase in dietary crude protein (from 25 to 35 %) in low protein:high non-protein energy diets positively affected weight gain whereas significantly (P <0.05) reduced weight gain was observed for a 10 % crude protein increase (from 45 to 55 %) in high protein:low non-protein energy diets. The whole body crude protein content was not affected by diet but the whole body crude lipid content decreased with the 10 % crude protein increase at each energy level. This study indicated a protein-sparing effect of non-protein energy sources in the diets of short-finned elvers. A lipid to carbohydrate ratio of 0.9 appeared to be needed for the maximum growth.

The optimum dietary digestible crude protein (DCP):digestible energy (MJ DE) requirement of the short-finned elvers was investigated with 7.5 % crude protein increments (from 25 to 55 % of the diets) in iso-energetic diets. The optimum dietary digestible crude protein was estimated as dietary percentage (% CP DM) and as a dietary digestible crude protein:digestible energy ratio (g DCP/MJ DE) using total weight gain (g). Second order polynomial (quadratic) and 5-SKM (five parameter saturation kinetics) models were chosen for the estimation of the optimum dietary

digestible crude protein. Two models gave similar results estimating the optimum dietary digestible crude protein as 43.0 % CP DM (±3.5) (r^2 = 0.79; F=9.2157; P=0.021) and 41 % CP DM (r^2=0.77) or as 24.5 g.DCP/MJ DE (±1.7) (r^2=0.83; F=12.0573; P=0.012) and 23.5 g DCP/MJ DE (r^2=0.75) respectively. Whole body crude protein and lipid contents tended to increase with increasing dietary crude protein in this experiment.

Nitrogen losses are primarily through faeces and metabolic excretion and largely influenced by dietary composition. Increasing protein content at two energy levels caused peak nitrogenous excretion rates 4-8 h following both the morning and afternoon feed. Daily ammonia-nitrogen excretion was significantly (P<0.05) higher on high protein:low non-protein energy diet (P55) compared to the P35 and P45 diets. Increasing dietary crude protein intake resulted in increasing ammonia- (y=0.022x+0.058; n=12; r^2=0.88; P<0.001) and urea-nitrogen (y=0.0044x+0.426; n=12; r^2=0.55; P<0.05) excretion in treatments. The proportional increase in urea-nitrogen excretion to total nitrogenous excretion with increasing dietary non-protein energy sources also indicated that urea-nitrogen excretion in the Australian short-finned eel could be more responsive to nutritional variables.

Fish meal is an expensive component of fish feeds and replacement of it with alternative protein sources without compromising the growth rate has been a priority in aquaculture nutrition research. Apparent digestibility coefficients were measured in order to assess the suitability of selected Australian plant and animal protein sources for fish meal replacement. Apparent crude protein digestibility was high for the selected Australian plant proteins and animal by-products. However, dry matter and energy digestibilities were found to be significantly (P<0.0001) higher for animal by-products than for plant proteins except for corn gluten meal. This was explained by the higher content of nitrogen free extract (NFE) in all the plant proteins except corn gluten meal. A final experiment was conducted to test the effects of fish meal replacement with corn gluten meal, meat meal, lupin meal and soy bean meal in diets using the optimum DCP/MJ DE ratio on growth, growth efficiencies and nitrogenous excretion by the Australian short-finned elvers by formulating the diets according to the optimum DCP/MJ DE ratio and ADC values determined in the present study.

This study primarily showed better dietary protein retention efficiencies when juvenile Australian short-finned eel were fed low protein:high non-protein energy diets and established the optimum DCP/MJ DE requirement of this species for maximum growth. The establishment of the optimum protein:energy ratio and identification of highly digestible alternative protein sources provides a basis for decreasing nitrogenous waste production, maximising protein retention and better eel culture practises in Australia generally.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS USED IN THE TEXT

Abbreviation	Full term
ADC	Apparent Digestibility Coefficient
ATP	Adenosine Tri Phosphate
BM	Blood Meal
BW	Body Weight
CGM	Corn Gluten Meal
CM	Canola Meal
CP	Crude Protein
d	day
DCP	Digestible Crude Protein
DE	Digestible Energy
DM	Dry Matter
DO	Dissolved Oxygen
EAA	Essential Amino Acid
FER	Feed Efficiency Ratio
FPM	Field Pea Meal
GE	Gross Energy
h	hours
l	litre
LM	Lupin Meal
min	minutes
MM	Meat Meal
NA	Not Applicable
NFE	Nitrogen Free Extract
ns	not significant
NSW	New South Wales
OUC	Ornithine Urea Cycle
PER	Protein Efficiency Ratio
PM	Poultry Meal
PPV	Productive Protein Value
SBM	Soybean Meal

SD	Standard Deviation
SEM	Standard Error of the Mean
SGR	Specific Growth Rate
5-SKM	Five Parameter Saturation Kinetics Model
WG	Weight Gain

CHAPTER ONE

General Introduction

1.1. The Australian short-finned eel

The eels are members of a tropical genus, *Anguilla,* and spend part of their lives in the sea and part in fresh water (Usui, 1974; Degani and Gallagher, 1995). Eighteen species of the genus *Anguilla* have been distinguished world wide, two of which, the European *A. anguilla* (Linnaeus) and American eels *A. rostrata* (Le Sueur), are Atlantic eels, while the rest are Indo-pacific species (Usui, 1974; Tesch, 1977; Degani and Gallagher, 1995). The distribution of various species of eels is shown in Table 1.1.

The southern hemisphere species are: *A. megastome* (Kaup), *A. reinhardtii* (Steindachner), *A. australis australis* (Richardson), *A. australis schmidti* (Phillips) and *A. dieffenbachi*. *A. megastome* (Kaup) is distributed from the Solomon Islands southwestward to the New Hebrides, New Caledonia, Fiji, the Society Island and Cook Island as far south as the Pitcairns (Ege, 1939). *A. reinhardtii* and *A. australis australis* mainly belong to the Australian continent. The Australian short-finned eel, *Anguilla australis australis* is one of the most important eel species in the southern hemisphere and occurs in Southeast Australia, from Cape Byron to Warrnambool as well as in Tasmania and Lord Howe Islands (Table 1.1) and is distributed along the greater part of the coasts of N.S.W and Victoria (Ege, 1939).

In Tasmania the Australian short-finned eel inhabited most Tasmanian coastal streams including those of King and Flinders islands and as far as 150 km inland in the major river system (Sloane, 1984d). However, the species was not recorded from the western Central Plateau, despite extensive sampling, with a single specimen from the River Ouse below Lake Augusta representing the farthest inland record. Few individuals of the Australian short-finned eel were found in the Great Lake but this species was common in other lakes in the Central Highlands at elevations of less than 1000 m (Sloane, 1984d).

Table 1.1. Distribution of the various species of the genus *Anguilla* (after Degani and Gallagher, 1995).

Species	Region	Distribution
A. megastoma (Kaup)	Southeast to south Pacific	Northeastward
A.reinhardtii (Steindachner)	" "	E.coast of Australia (Northern N.S.W and Southern Queensland)
A. australis australis (Richardson)	" "	Southeastern Australia (From Cape Byron to Warrnambool, Tasmania and Lord Howe Island)
A. australis schmidti (Phillips)	" "	New Zealand
A. dieffenbachi (Gray)	" "	New Zealand
A. bicolor bicolor (Schmidt)	Pacific, Equator	Insular, east part
A. interioris (Whiley)	" "	New Guinea
A. b. bicolar (McClelland)	North to equatorial Indian Ocean	Northward
A. nebulosa nebulosa (McClelland)	"	Sri Lanka, India Burma
A. anguilla (Linneaus)	North Atlantic	Europe, N. Africa
A. rostrata (Le Sueur)	" "	North and South America
A. japonica (Temmink, Schlegel)	Northeast Pacific	Japan and China

The elver catches in Tasmania indicated that many individuals spend several years in the upper estuarine areas and some probably spend their entire freshwater feeding phase there (Sloane, 1984c). Moreover, very few were captured more than 6 km from tidal influence. This indicates very limited movement upstream with most elvers migrating only short distances into freshwater in the first two years. In the next stage, migration further inland , the size and age of elvers increased and the length and weight ranges of elvers sampled widened (Sloane, 1984c).

1.1.1. Life cycle and feeding in the wild

The most important feature of the eel life cycle is their catadromous migration towards spawning grounds in the sea (Usui, 1974; Tesch, 1977; Jellyman, 1974, 1979; Tsukamoto, 1990; Lee Chen and Chen, 1991; Ciccotti et al., 1995; Degani and Gallagher, 1995; Kruse et al., 1996). Specifically the European eel (*A. anguilla*), in regard to distance, covers the longest distance among other eel species and travels 7000 km from the inland waters of Europe to the spawning ground in the Sargasso sea (Usui, 1974; Tesch, 1977; Degani and Gallagher, 1995). Unfortunately, mapping the size of distribution for the leptocephali of south western Pacific eels at sea in order to determine the likely breeding areas has not been possible due to the paucity of larval collections (Castle, 1963). However, Sloane (1984b) concluded that glass eels of the Australian short-finned eel entering Tasmanian streams are larger than in New South Wales and may indicate that Tasmania is more distant from the breeding grounds (presumed off the coasts of Vanuatu) and the larvae are probably distributed by the East Australian Current.

After spawning , leptocephali are presumed to drift on major ocean currents and metamorphose into glass eels before entering freshwater (Tesch, 1977). Upstream migration begins when glass eels are attracted to natural odours in freshwaters (Usui, 1974; Tesch, 1977; Jellyman, 1974; Degani and Gallagher, 1995). As eels migrate upstream they become pigmented and are referred to as elvers (Degani and Gallagher, 1995). When pigmentation is complete the yellow eel stage is reached and there are no further major external changes until the eels return to the sea (Usui, 1974; Tesch, 1977; Degani and Gallagher, 1995). Eels returning to breeding grounds are called silver eels (Usui, 1974; Tesch, 1977; Degani and Gallagher, 1995).

Information on the nutritional physiology and natural feeding habits of eels in the wild is important for the development of artificial diets and feeding regimes that will meet both nutritional and feeding requirements in culture conditions. The eel's diet changes during its life history. During metamorphosis from leptocephalus to glass eel no feeding occurs. Thus, the initiation of feeding in glass eels is one of the most difficult parts of eel culture. For example, when glass eels reach the continental shelf of Europe, they have empty intestines and little food is found in those with grey pigmentation (Eichelbaum, 1924 cited in Degani and Gallagher, 1995). Food intake appears to correlate with increased pigmentation (Degani and Gallagher, 1995).

During migration to the inland waters, one must assume that the leptocephalus consume substantial amounts of food to meet their energy requirements for migration and development. However, information on the feeding ability of leptocephalus has been limited due to difficulty in sampling. Nevertheless, the leptocephalus are assumed to feed on plankton (Degani and Gallagher, 1995). However, it is not certain whether the feeding activity is active or passive (filtering). Kurokawa et al. (1995) showed that six days after hatching an antibody to eel trypsinogen showed weak binding in the pancreas, suggesting that the leptocephalus pancreas synthetises digestive enzymes. The immunohistochemical response became stronger at 7-d post hatch at which time the mouth opening orientation moved from ventral to anterior, the intestine differentiated into small intestine and rectum and the yolk was absorbed. Rotifers were also first observed in the digestive tract of 13-d old artificially produced Japanese eel larvae (Kurokawa et al., 1995). Kruse et al. (1996) had also been able to identify the presence of trypsin in intestinal structures of entire European eel larvae and further localised to distinctive areas of the digestive tract by anti-eel trypsin immunohistochemistry. These results suggested that digestive system in the larvae has the capacity to degrade macromolecules (Kurokawa, 1995; Kruse et al., 1996).

Natural feeding of Southern Hemisphere eel species has been mainly investigated in Australian and New Zealand eel species in freshwater (Beumer, 1979; Sloane, 1984a; Jellyman, 1989). Beumer (1979) studied the feeding habits and movement of the Australian short-finned eel in Macleods Morass, Gippsland, Victoria, Australia. The Australian short-finned eel, like other eel species, is

considered carnivorous and consumes mainly protein and lipid rich prey items (Lecomte-Finiger, 1983). Insects were found to be the major prey for the Australian short-finned eel. No relationship between size of prey ingested and size of eel was evident. Similarly, Sloane (1984d) found that the diet of the Australian short-finned eel changed significantly in the Douglas River, Tasmania. Fish were not a major food of larger individuals (≥ 90 cm) and the diet of small (≤ 20 cm) eels was predominantly Ephemeroptera, Diptera and Trichoptera (Sloane, 1984d).

1.2. Eel farming in Australia and around the world

Although eel farming is relatively new to Europe, North America and Australia, the raising of eels under more or less controlled conditions of aquaculture has been carried out in Japan since 1876 (Tesch, 1977; Arai, 1991; Degani and Gallagher, 1995). There are a number of reasons why eel culture has developed so rapidly during this century. The main one being higher demand from mostly Asian and Northern European countries. Japan, China, Denmark, Netherlands and Germany are the biggest consumers of eels (Seymour, 1984; Heinsbroek, 1991; Ikenoue and Kafuku, 1992; Degani and Gallagher, 1995). A decrease in the natural population of eels due to the pollution of rivers and high prices in Europe and Japan have also contributed to eel farming as a viable commercial option (Belpaire, 1990; Degani and Gallagher, 1995).

Over 100.000 tonnes of Anguillid eels, mainly European and Japanese, are cultured annually around the world. More than 70% of cultured eels comes from Japan and Taiwan (Arai, 1991). Eel culture methods vary depending on the climate of the region, available technology and finance. However, in recent years traditional culture techniques (earthen ponds, farming using large quantities of water produced by power plants etc.) gave way to temperature controlled closed recirculating systems. These reduce large temperature fluctuations, prevent water being too cold in winter and too warm in summer time and therefore addressed reduced growth rates and induced stress and bacterial diseases (Belpaire, 1991).

Eel farming in Australia is in its early stages. Farms are being set up in Tasmania, Victoria, New South Wales and Queensland. However, these farms are

still in their developmental stage. The long-finned eel, *A.reinhardtii* makes up the majority of the catch in Queensland and the large size of this species is preferred by Asian markets (mainly in Hong Kong) for live eel (Zeller and Beumer, 1996). The Australian short-finned eel is the most important commercial species in Victoria and Tasmania and is marketed as processed product to Europe (Zeller and Beumer, 1996). Australia can play a major role in supplying the demand both for live larger eels (the long-finned eel) from parts of Asia and processed (smoked and frozen fillets) cultured eels (the Australian short-finned eel) from the European countries since the depletion of wild stocks is a major concern for other eel producing countries (Zeller and Beumer, 1996). However, information about the physiological, environmental and nutritional requirements of the Australian short-finned eel in a controlled aquaculture systems is very limited. In order to establish an efficient eel culture in Australia, environmental, nutritional and physiological requirements of short-finned eel should primarily be addressed.

1.3. Protein nutrition of eels

The relationships between dietary composition, water quality and growth are set to be the most important aspects of the development of intensive eel culture in Australia. In this regard, reduction of ammonia excretion, minimisation of accumulation of organic matter and efficient biofiltration in high density eel recirculating culture systems are crucial in order to maintain good water quality. These issues can be addressed through the development of optimum dietary and feeding regimes.

1.3.1. The optimum dietary protein requirement

Fish require diets with 35-55% protein to reach maximum growth rate compared to 12-25 % required by mammals and birds (Bowen, 1987). Because protein is a major and expensive component of fish feeds, determination of the optimum level that produces maximum growth is the obligatory first step in feed formulation. Although there is no published material on the nutritional requirements of the Australian short-finned eel, considerable research on protein requirements of the Japanese, European and American eels has been completed (Nose and Arai, 1972;

Degani et al., 1985;1987; Tibbetts et al., 2000).

Expressed as a percentage of diet dry weight, the concentration of protein in the diet which produces maximum growth has been the most frequently used measure of protein requirement for growth of fish species (Pandian and Vivekanandan, 1985). However, these observations are relative measures and closely related to the non-protein energy sources provided in diets. Since fish do not have a requirement for protein *per se* but rather require a balance of amino acids (Wilson and Halver, 1986), it is better to express the protein requirement on a digestible basis and in relation to energy intake: g.digestible dietery crude protein to digestible energy (g.DCP/MJ DE). Requirements determined in these terms may differ because the species studied use protein differently, have different physiological growth potentials, held under different dietary (different protein source and feeding regimes), temperature or photoperiod conditions or due to some combination of these variables (Bowen, 1986). Availability of amino acids and energy sources used in experimental diets are also given as likely to explain a considerable part of the differences observed in protein requirement for eels (Degani et al., 1985; 1987). The optimum protein requirements of *Anguillid* eels have been determined in several studies and show considerable variation (see Table 5.5).

1.3.2. Dietary energy sources for eels

The biggest difference in nutrition between fish and endothermic animals is that fish have a lower energy requirement due to their lower maintenance requirements, lower energy requirement for nitrogen excretion and lower requirement for maintaining posture (Page and Andrews, 1973; Lovell, 1989). Because energy needs for maintenance and voluntary activity must be satisfied before energy is available for growth, dietary protein will be used for energy when the diet is deficient in energy in relation to protein (Cho and Slinger, 1982; Lovell, 1989). Compared to proteins, carbohydrates and lipids are less expensive forms of dietary energy yielding ingredients for fish species (García-Gallego et al., 1994; Wilson, 1994). However, their utilisation by fishes varies and remains an area where considerable research is required. In order to create well balanced and cost effective aquaculture feeds, fish nutritionists have concentrated on not only the replacement of fish meal (most

commonly used expensive energy source in fish diets) with other alternative cheaper protein sources but also on the metabolism and utilisation of carbohydrates and lipids (De la Higuera et al., 1977; Cho and Kaushik, 1985; Degani and Viola, 1987; García-Gallego et al., 1994, 1995;). The optimum utilisation of carbohydrates and lipids in diets will allow the majority of the protein in the diet to be retained by the fish. An imbalance in the protein/energy ratio will lead to either wastage of protein by satisfying maintenance energy requirement before growth or reducing feed consumption and thus lowering the intake of the necessary amount of protein and other essential nutrients for maximum growth (NRC, 1993; De Silva and Anderson, 1995). Lower than optimum the protein/energy ratios in diets have been demonstrated to increase ammonia production in the American eels, (Gallagher and Matthews, 1987). Excessively high energy to protein ratios in fish diets can also lead to deposition of large amounts of body fat which can be undesirable in food fish (NRC, 1993).

Although eels are carnivorous (Lecomte-Finiger, 1983), they utilise dietary carbohydrate more efficiently than some other carnivorous fish species (Degani et al., 1986; Degani, 1987; Degani and Gallagher, 1995; García-Gallego et al., 1991, 1994, 1995; Hidalgo et al., 1993; Sanz et al., 1993). However, temperature, fish size and the source of fats and carbohydrates must be taken into account. Spannhof and Kuhere (1977) found that eels digest potato starch better than trout do. García-Gallego et al. (1995) also showed that the European eels have a comparatively greater ability of using diets with a high level of corn starch for growth, food conversion and utilisation of dietary protein and energy than rainbow trout (*Oncorhyncus mykiss*). Recently Birkett (1996) showed that the Australian short-finned eel utilised up to 30 % carbohydrate (gelatinised potato starch) without showing any adverse growth effect compared to controls fed with low carbohydrate and very rich protein diet. It appears that eels adapt to a high carbohydrate diet and convert the excess energy into lipids much more efficiently in carbohydrate utilisation than those fish that lack that ability (Degani, 1987a).

Lipids contain more energy per unit weight than the other major nutrient components and they are used efficiently by fish as an energy source (Brafield, 1985). However, their use in fish feeds is governed by the maximum inclusion levels

which different species can tolerate. Lipids play an important role in the diets of eels. Eels store fat during growth (Gallagher et al., 1984 a,b), probably for use as an energy source during migration to the breeding ground. In that sense, lipids are important both for achieving significant protein economy and the continuation of other vital physiological mechanisms in eels. Improvements in protein utilisation have both been shown for the European eels and trout, judged by an increase in biological value and a significant increase of protein productive value, when maximum tolerable levels of lipids were included in their diets (De la Higuera et al., 1977; Dosoretz and Degani, 1987).

1.3.3. Metabolic excretion as a function of dietary composition

Ammonia is the major end product of nitrogen metabolism in teleosts and mainly influenced by dietary composition (Forster and Goldstein, 1969; Wood, 1993; Korsgaard et al., 1995). A small but significant proportion (5-15%) of waste nitrogen is also excreted as urea (Brett and Zala, 1975; Kaushik, 1980; Kaushik and Cowey, 1990). The excretion of other nitrogenous end products or metabolites like uric acid, creatine, taurine, purines, imidazole and trimethylamine oxide by teleosts is quantitatively small and their contribution to total nitrogen excretion is limited (Kaushik and Cowey, 1990; Walsh, 1998). In growing fish, absorbed amino acids in excess of those needed for protein synthesis are deaminated and excreted as TAN (total ammonia nitrogen) (Wood, 1993, Houlihan et al., 1995a,b). Even those amino acids that are used for protein synthesis may subsequently be returned to the free body amino acid pool and excreted (Garlick et al., 1994). Besides the dietary composition, the biological value or the quality (availability and the balance of amino acids) of a given dietary protein is the most important determinant of the amplitude of nitrogenous excretion in fish (Kaushik and Cowey, 1990).

Compared to ammonia, *de novo* synthesis of urea is energetically costly to teleosts requiring an input of at least 2.5 ATP per nitrogen excreted (Korsgaard et al., 1995; Walsh, 1998). Increasing body of evidence suggests that there might be a dietary induced alkalosis and ureogenesis in fish (Jayaram and Beamish, 1992). Highly variable urea-nitrogen and increased urea-nitrogen with increased feed intake have been reported with several fish species (Kikuchi, 1995; Harris and Probyn,

1996; Carter et al., 1998; Verbeeten et al., 1999). It also appears that diets high in lipids increase the proportion of urea-nitrogen in the total nitrogenous excretion of fish species (Jayaram and Beamish, 1992). Increased urea- and reduced ammonia-nitrogen excretion in fish fed high lipid diets probably reflect the involvement of dietary fatty acids in sparing the deamination of amino acids and increasing the pool of amino acids for ureogenesis in the liver (van den Thillard, 1986) since reduced glutaminase activity has been demonstrated with several fish species fed such diets as ammonia being an end product of the metabolism of glutamine to glutamate (Walsh and Milligan, 1995; McGoogan and Gatlin, 1999). Therefore, it is of interest to investigate the effects of nutritional variables on nitrogenous excretion in order to examine constraints on the maximum utilisation of dietary nitrogen for growth.

1.4. Feed development studies for eels

Fish meal replacement

Feed cost accounts for 60-70% of fish farming operational costs. A major issue in aquaculture nutrition is the use of fish meal and the realisation that it is a finite resource (Welcomme, 1996; Hardy, 1997). Each fish produced by aquaculture requires the capture of four, or more, times its weight of marine fish to produce the fish meal for the feed (Hardy, 1997). Fish meal is one of the most expensive ingredients of fish feeds (Naylor et al., 2000). Thus, it is potentially beneficial to reduce or completely replace fish meal with cheaper alternative protein sources.

Animal by-products (blood meal and poultry meal), fish silage, soybean meal, meat meal and sunflower meal have been trialed as an alternative protein sources in eel diets (Degani et al., 1985; Degani, 1987b; Gallagher and Degani, 1988; Gonçalves et al., 1989; Lee and Bai, 1997; García-Gallego et al., 1998). Gallagher and Degani (1988) found that poultry meal could be used as long as it was not the only source of protein in diets of the European eel (2.1 ± 0.2 g mean weight). Previous studies by Degani et al. (1984) and Degani (1986) also showed that no significant change in growth of European glass eels when 50% of the dietary fish meal was replaced with poultry meal.

Fish silage and blood meal also been tested for fish meal replacement in juvenile European and Japanese eels (Gonçalves et al., 1989; Lee and Bai, 1997). Gonçalves et al. (1989) replaced fish meal with increasing (10, 15 and 20%) percentages of protein from fish silage in isocaloric diets. The incorporation of silage in the diet resulted in increments in the SGR (Specific Growth Rate, %/d), the FER (Feed Efficiency Ratio) and the PER (Protein Efficiency Ratio) when compared to a control population fed only fish and meat meal, as protein source during a 4-month period. No differences were seen in the effectiveness of the diets incorporating different percentages of fish silage. Greater food intake, probably due to a different texture of the food or the presence of food attractants, were given as likely reasons for the increase in growth rate and the presence of greater amounts of lipids in the carcasses (Gonçalves et al., 1989). According to Lee and Bai (1997) blood meal could also be substituted for fish meal in juvenile Japanese eel diet at up to 50% without supplementation of three essential amino acids (arginine, isoleucine and methionine) and up to 75% with three EAA supplementation. Lysine, threonine, methionine and histidine supplemented sunflower meal was found to replace all the fish meal in the European eel diet without compromising the growth rates (García-Gallego et al., 1998).

Plant proteins are not utilised as effectively as animal proteins because they may contain several anti-nutritional factors and a high carbohydrate content or need essential amino acid supplementation (Jobling, 1994). For instance, soybeans contain protease inhibitors, lectins, goitrogens, antivitamins, phytases, saponins, various oligosaccharides and antigenic proteins (allergens) (Jobling, 1994; Rumsey et al., 1995). However, for the elimination of such anti-nutritional factors feed manufacturers take advantage of the recent advances in the heat treatments of raw materials (eg. extrusion, cooking, toasting of soybeans) or use genetically improved varieties of plant materials (eg. low glucosynolate-containing rapeseed, low alkaloid lupin) (Lovell, 1989; Kaushik, 1990; Mitchell et al., 1991; Jobling, 1994; Rumsey et al., 1995). Although these treatments may influence the digestibility of the final product and considerable care is required in the choice of processing methods. Such technologies generally improve the use of plant proteins (Gouveia et al., 1993; Olli et al., 1995; Pfeffer et al., 1995).

Although soybean meal is frequently included as a major protein source in dry feeds for species such as channel catfish *Ictalurus punctatus*, grass carp *Cytenopharyngodon idella* and milkfish *Chanos chanos*, its use in diets for some carnivorous fish species like salmonids and eels may be somewhat limited due to phytic acid and high levels of oligosaccharides in soybeans (Jobling, 1994). Phytic acid can reduce the availability of minerals and the protein digestibility of the diets. However, the addition of microbial phytase to the diets has been reported to improve the utilisation of phytate phosphorus by rainbow trout (Cain and Garling, 1995; Rodehutscord and Pfeffer, 1995). Although high levels of oligosaccharides in soybeans may be harmful and halts the growth rates for some carnivorous fish species, eels can tolerate higher carbohydrate levels better than other carnivorous species (García-Gallego et al., 1995) and this may justify the use of soybeans as alternative protein source in their diets. Eels (*A. anguilla*) were fed 0, 10, 20% soybean meal in their diet and an increase in the percentage of soybean protein at the expense of animal protein (fish and poultry meal) decreased the growth rate of the eels (Degani, 1987b). However, the results of this experiment could have been different if the anti-nutritional factors of soybeans had been eliminated by pre-treatments of diets such as the addition of microbial phytase.

Fish meal is the most commonly used protein source in eel feeds. The suitability of a protein source for fish meal replacement in fish feeds is a measure of its protein and energy digestibility or physiological value to species. The digestibilities of feed ingredients and diets used in fish farming are related to the nutritional characteristics of the raw materials, manufacturing process and digestive capabilities of fish as well as to the experimental techniques employed for faecal collection (Tacon, 1990; Gomes et al., 1998). Little is known about the digestibility of alternative protein sources for the Australian short-finned eel (De Silva et al., 2000) and this has probably restricted trials to assess the effect of fish meal replacement on growth and growth efficiencies. Therefore, proper measurement of apparent digestibility coefficients of a protein source is a key component of fish meal replacement trials and needed to be addressed for successful feed development studies with the Australian short-finned eel.

1.5. Aims of the present study

This study aimed at investigating the aspects of protein nutrition in the juvenile Australian short-finned eel in order to understand dietary induced changes in nitrogen metabolism and use the information for effective dietary formulation in Australia. The approach taken was to assess: a) the effects of non-protein energy yielding substrates on growth and growth efficiencies in diets with increasing protein contents at two different energy levels, b) the optimum dietary digestible protein to digestible energy ratio as g.DCP/MJ DE, c) 24-h cycles of nitrogenous excretion in response to increasing dietary crude protein at two different energy levels, d) the biological value of several Australian plant and animal proteins measured by their apparent digestibility coefficients for dry matter, crude protein and energy and e) the effects of 23% replacement of fish meal protein with corn gluten meal, soybean meal, lupin meal and meat meal on growth and growth efficiency (including daily nitrogenous excretion and apparent digestibility coefficients) of juvenile Australian short-finned eel in balanced diets (the diets were formulated according to optimum digestible crude protein to digestible energy required for a maximum growth in the Australian short-finned eel demonstrated earlier in this project).

Chapters 3 and 4 respectively, cover different aspects of two experiments and contain some repeated information in the introduction and materials and methods sections. Several chapters are in preparation for publication with one published as described below.

Chapter 4; Engin, K., Carter, C.G., 2001. Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level. Aquaculture 194, 123-136.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

2.1. Fish and maintenance

Elvers of the Australian short-finned eel (*Anguilla australis australis*) used in this study were supplied by Inland Fisheries Commission, Tasmania. Elvers were caught using fyke nets from two different localities in Tasmania. Three batches of elvers were used in the entire study. The first and third batch of elvers were captured from the North Esk River (41°S 147°E) in North Eastern Tasmania in the upstream migration seasons (from December to April) of 1997 and 1999 respectively. The second batch of elvers, however, were captured from the Derwent River (43 °S 147 °E) in Southern Tasmania in April 1998 due to low occurrence in the North Esk River.

Following arrival, elvers were placed in nine 360-l round fibreglass tanks in a recirculating system. Before putting the elvers into tanks, they were chlorinated and rinsed thoroughly with freshwater. This system was used as the holding system for elvers until experimentation. Aerated city water was used and the water temperature thermostatically maintained at 24±1 °C all year round.

Before being used in experiments, elvers were weaned on to a commercial eel diet (Chinda Entreprise Corp., Taiwan) in the holding system. The powdered commercial eel diet was mixed with frozen blood worms (*Tubifex* spp.) and water in a ratio of 1:1:1 and resultant mash fed to the elvers at 6 % BW/d (percent body weight per day). The mash was placed into the side of perforated plastic tubs through which elvers could access the feed easily. The amount of *Tubifex* was reduced gradually after two weeks of feeding and completely replaced with the commercial food at the end of the fourth week. Except for the first batch of wild stock, the second and third batches housed in the holding system were fed with pelleted (1mm die) commercial diet (Chinda Enterprise Corp., Taiwan) after weaning.

Randomly selected elvers from the holding system were anaesthetised (80 mg/l, Benzocaine), individually weighed to the nearest 0.01 g, length measured to the nearest 0.1 mm and randomly distributed to tanks in the experimental culture system (see below).

2.2. Culture systems

Experiments were carried out in a freshwater recirculating culture system which consisted of 19.8-l blue translucent carboy tanks (Figure 2.1a). Black mesh cloth was placed under the tank lids in order to prevent elvers escaping. Initially the system had twelve tanks (Chapter 3 and 4). However, for later experiments (Chapters 5, 6 and 7), a further 3 tanks were used. The digestibility trial (Chapter 6) was conducted using a separate 3-carboy recirculating system designed as a modified settlement collector (Cho et al., 1982) (Figure 2.2). Both systems were housed in a temperature and photoperiod controlled room.

Freshwater was recirculated through a submersible pump (Eheim 1060, Germany) in the system (Figure 2.1a). A constant water flow of 1.1 l/min. into each carboy tank was maintained with ¾" valves. 32 mm PVC pipes covered with dacron mesh was utilised to maintain water level stable in each carboy tank (Figure 2.1b). Outflow of water from the carboys was directed to a main 50 mm PVC pipe with series of 25 mm PVC pipes, elbows and plastic tubing. The main 50 mm PVC water outflow collector was positioned gravimetrically in the system so that the outflow of water into main sump was maintained on a certain pace (Figure 2.1a). Biofiltration was made by trickle tray biofiltration units in both system. Trays containing plastic bioballs were perforated on the bottom and installed on top of a series of containers similar sized to a main sump (Figure 2.1a). Submersible pumps located in each container continuously pumped the water to the top of the trays from where it trickled down back into the containers. Water was sprayed over the trays with 25 mm PVC pipes prepared as spray bars. An adjacent 1000-l fibreglass tank was used as an aerated freshwater reservoir for both systems and water exchange was done manually into the sump. The reservoir was kept full with city water throughout the study and used only at ambient temperature.

2.3. Water quality analyses

Water temperature was measured daily whereas DO (dissolved oxygen), pH, ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N) were measured twice

a week in three randomly selected tanks. An OxyGuard Handy MK III oxygen

Figure 2.1a. Schematic overview of the recirculating culture system. ⟶ indicates the direction of inflowing water whereas → indicates the direction of outflowing water in the recirculating system.

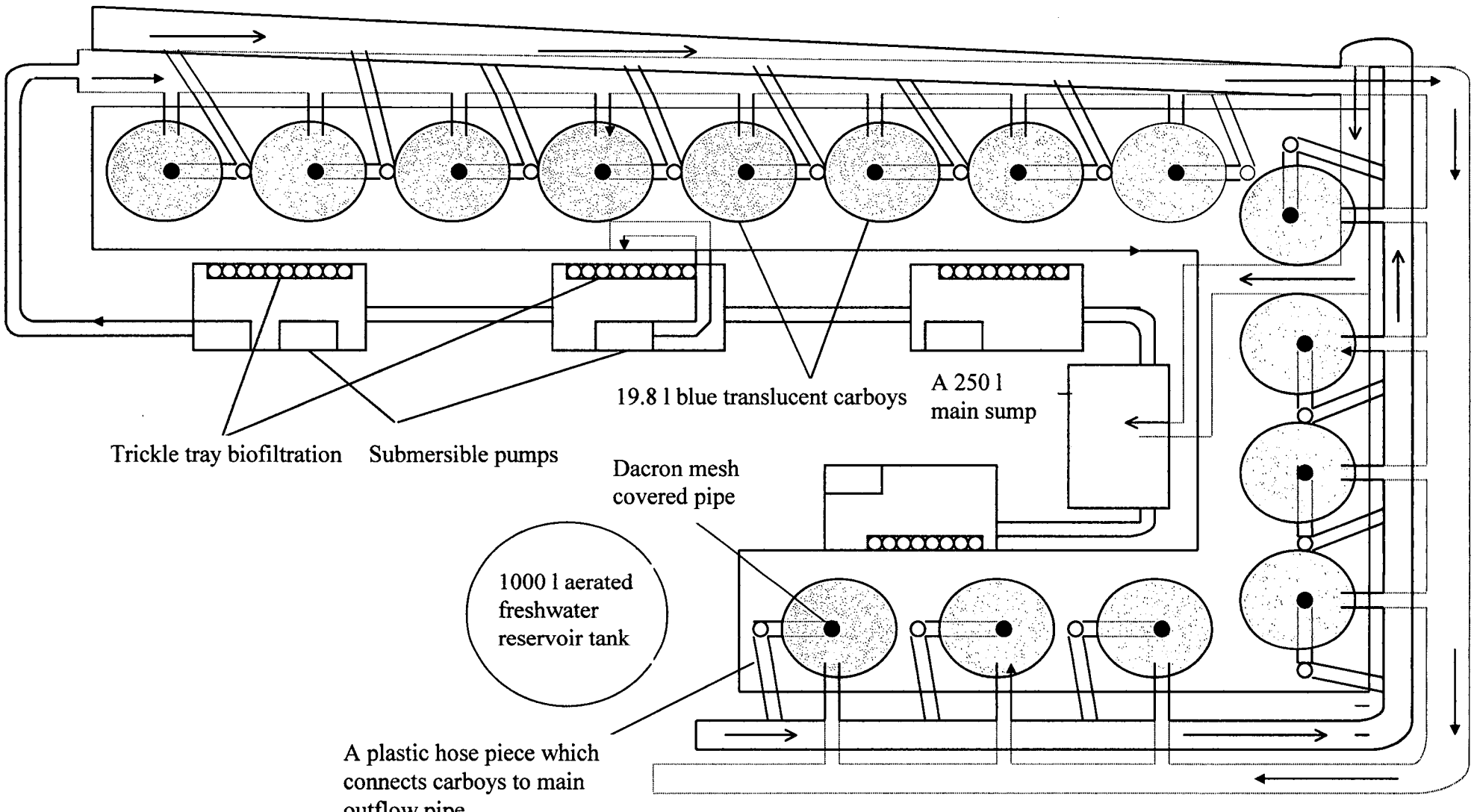
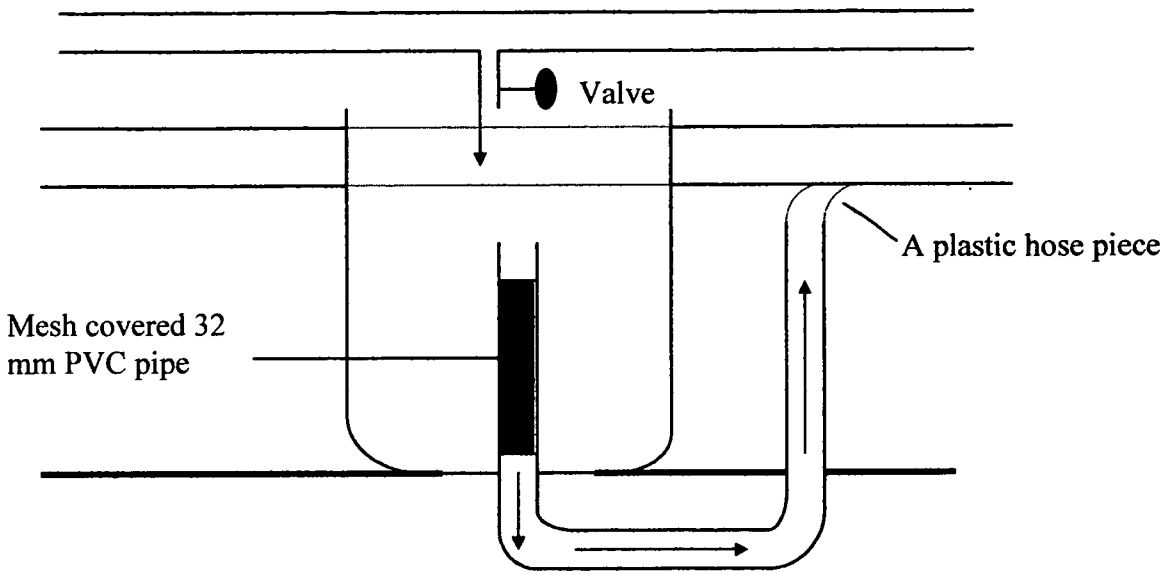

19.8 l blue translucent carboys
A 250 l
main sump
Trickle tray biofiltration
Submersible pumps
Dacron mesh
covered pipe
1000 l aerated
freshwater
reservoir tank
A plastic hose piece which
connects carboys to main
outflow pipe

Figure 2.1b. Schematic side view of an individual carboy tank. ——▶ indicates the direction of the water flow in the system.


Valve
A plastic hose piece
Mesh covered 32
mm PVC pipe

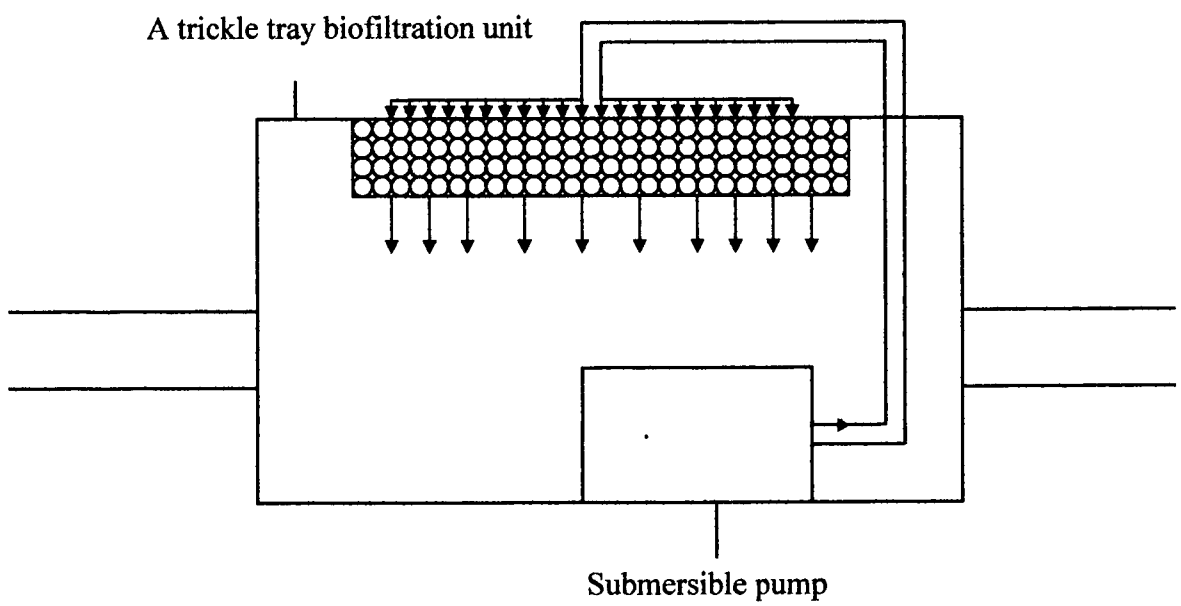
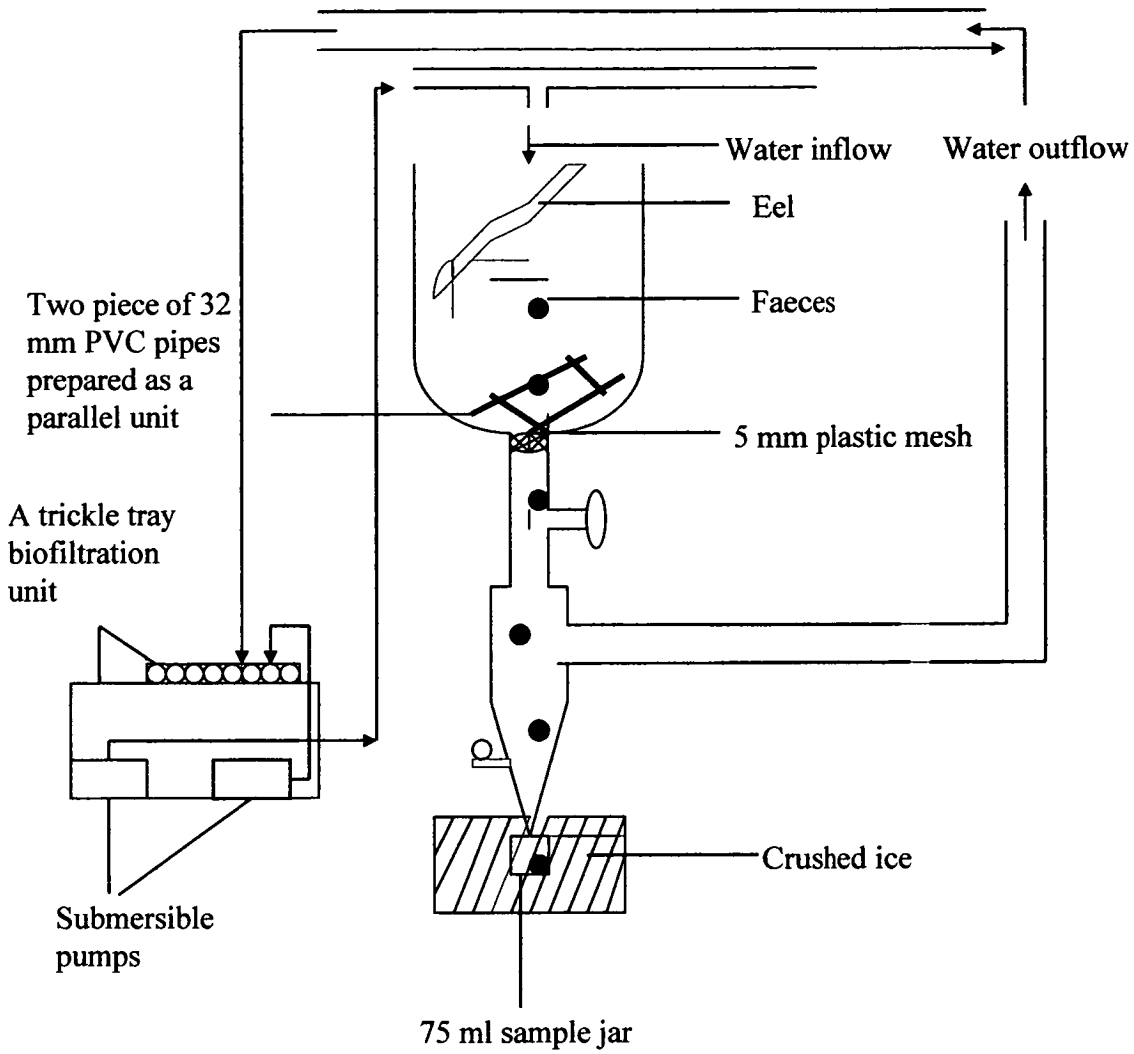

A trickle tray biofiltration unit
Submersible pump

Figure 2.2. Schematic side view of the digestibility system used in the measurement of digestibility coefficients of selected ingredients in the Australian short-finned eel.


Water inflow
Water outflow
Eel
Faeces
Two piece of 32 mm PVC pipes prepared as a parallel unit
5 mm plastic mesh
A trickle tray biofiltration unit
Crushed ice
Submersible pumps
75 ml sample jar

electrode, calibrated in air-saturated freshwater, was used for DO measurements. The pH was measured using a pH meter and combination glass electrode (Hanna Instruments, model HI 9017). The pH meter was calibrated with phosphate (pH=7.00) and borate (pH=9.20) buffers before use. Ammonia-nitrogen (NH_3-N) and nitrite-nitrogen (NO_2-N) were measured with salicylate and diazotisation methods respectively (HACH, Company, Colorado, U.S.A).

Methods for measuring ammonia- and urea- nitrogen excretion are described in Chapters 4 and 7.

2.4. Feeding and food consumption

A fixed ration of 5 and 6 % BW/d was employed throughout this study. Elvers were fed twice every day between 0900-1000 h and 1700-1800 h. Feed was presented as mash in two experiments (Chapters 3 and 4) and as pellets in subsequent experiments (Chapters 5,6 and 7). Before being used in experiments, elvers were trained to take pellets in the holding system using a pelletised commercial eel diet (see above). Feed consumption was measured by siphoning the uneaten food from each tank 20-25 min following morning and afternoon feeds. In the digestibility experiment uneaten feed was collected by flushing through the faecal collectors. Uneaten feed was then dried at 70 °C for 16 h (AOAC, 1995) and calculated as % BW/d. All tanks were checked daily for mortality and escapees were treated as mortalities in experiments. Mortality was below 10 % in all experiments.

2.5. Cleaning

The main culture system tanks were cleaned thoroughly every 7-10 d. However, dacron mesh covered pipes were cleaned using a pressurised garden hose every 2 d since accumulated uneaten feed and faeces blocked the drainage of water through the tank. Cleaning involved turning the water flow off the tanks, gently cleaning the bottom and edges of the tanks with a sponge and siphoning enough water from the tanks. Immediately after the siphoning the taps were turned on and the water flow to each tank resumed. This cleaning procedure took less than 5 min. for any tank and it was always done 3-4 h following feeding. The amount of water taken out of the

system was replenished manually into the main sump from the 1000-l freshwater reservoir.

Manufacture of experimental feed, chemical and statistical analyses are described in detail in the appropriate Chapters.

CHAPTER THREE

Growth and feed utilisation of the Australian short-finned eel, *Anguilla australis australis* (Richardson) fed with paired iso-energetic diets with increasing crude protein content

3.1. Introduction

The interaction between dietary protein and non-protein energy sources (lipids and carbohydrates) is complex due to dietary protein being part of dietary energy and the high energy requirement of protein turnover and deposition (Garling and Wilson, 1976; Boorman, 1980; Cho et al., 1982; Houlihan et al., 1995a,b; Steffens, 1996). Therefore, it is of interest to supply fish with a favourable dietary protein:energy ratio since meeting energy requirements mainly with protein is costly from both physiological and economical points of view (Ohta and Watanabe, 1996; Steffens, 1996; Engin and Carter, 2001).

High energy diets with highly digestible ingredients (low protein:high lipid:medium or high carbohydrate) were shown to result in rapid growth and an efficient feed conversion in several fish species (Page and Andrews, 1973; Cho and Kaushik, 1985; Dosoretz and Degani, 1987; Hillestad and Johnsen, 1994; Einen and Roem, 1997) including eels (García-Gallego et al., 1991). Channel catfish was shown to require less dietary protein and more dietary energy for per gram weight gain when fed increased dietary protein to energy ratio diets (Page and Andrews, 1973). Hillestad and Johnson (1994) demonstrated that Atlantic salmon smolt in sea cages grew 27% better when fed lower protein and higher lipid diets than those on higher protein and lower lipid diets. This is attributed to the protein-sparing effect of lipids and carbohydrates in fish feeds (Sanz et al., 1993; Steffens, 1996). Lipids were found to be main energy source which induces the protein-sparing effect in carnivorous fish species (Austreng, 1978; Tibaldi et al., 1996). Although eels are considered carnivorous (Lecomte-Finiger, 1983; Arai, 1988), nutritional studies with eels demonstrated that a dietary carbohydrate to fat ratio that is higher than considered optimum for other carnivorous fish species may be needed for eels to show a protein-sparing effect (Hidalgo et al., 1993; Sanz et al., 1993; García-Gallego et al., 1991;1995). Previous studies with the European eel demonstrated that the endocrine control of carbohydrate metabolism in the eel is similar to that in mammals and different to that operating in other carnivorous fish where insulin is more closely related to protein metabolism (Ince and Thorpe, 1974; Lewander et al., 1976). Changes such as hypoglycemia, depletion of hepatic glycogen and an initially increased

muscle glycogen formation were observed in insulin injected eels and qualitatively similar to those observed in mammals (Lewander et al., 1976). The varying role of insulin between fish species is probably partly due to both different evolutionary history and dietary habits (Thorpe and Ince, 1974; Lewander et al., 1976).

Insufficient dietary energy prevents full utilisation of dietary nitrogen and imposes a threshold on nitrogen retention (Boorman, 1980). In contrast, diets containing excess energy can reduce feed consumption and thus lower the intake of necessary amount of protein and other essential nutrients for maximum growth (NRC, 1993). There is little known about the nutritional requirements of the Australian short-finned eel (De Silva et al., 2000; Engin and Carter, 2001). This study aimed at demonstrating the effects of increasing dietary crude protein with two constant energy levels on the growth and feed utilisation of the Australian short-finned eel.

3.2. Materials and Methods

3.2.1. Fish and maintenance

Thirty elvers of the Australian short-finned eel (1.83±0.01 g average wet weight) were randomly allocated to twelve 19.8-l carboys incorporated into a recirculating system following one week of acclimation to diets and experimental conditions (Engin and Carter, 2001). During weight measurements elvers were anaesthetised (80 mg/l, Benzocaine). Uneaten feed and faeces were siphoned out daily throughout the experiment. Mean values (±SD) of water quality parameters were recorded throughout the experiment: 25±0.4 °C water temperature, 5.8±0.3 mg/l DO, 6.59±0.4 pH, 0.22±0.03 mg/l ammonia-nitrogen (NH_3-N) and 0.018±0.004 mg/l nitrite-nitrogen (NO_2-N).

3.2.2. Diets

Four experimental diets were formulated to contain 25, 35, 45 and 55% crude protein on a DM basis (Table 3.1). Diets were formulated to make the pairs P25

and P35 or P45 and P55 isoenergetic. Diets contained fish meal and fish oil from jack mackeral, *Trachurus picturatus* (Pivot Aquaculture Ltd., Tasmania, Australia), dextrin (Bunge Bioproducts, NSW, Australia) and the other ingredients by Sigma-Aldrich Pty.Ltd.(Australia). L-ascorbyl-2-polyphosphate (Stay-C, Hoffman La Roche Pharmaceuticals, Basil, Switzerland) was used with vitamin and mineral mixtures as described by De la Higuera et al. (1989). Diets were prepared by mixing dry ingredients in a food mixer for 25 min followed by fish oil for a further 30 min.

3.2.3. Experimental procedure

Elvers were fed twice a day between 0900-1000 h and 1700-1800 h on rations equal to 6% BW/d for 63 days. Before being fed, dry daily rations were mixed with water at a ratio of 1:1 and cut into two equal pieces. After the morning feeding, the remaining feed was stored at - 20 °C until the late afternoon feeding. Ration was adjusted every 3 weeks throughout the experiment following weighings when elvers were individually weighed and length measured in each weighings. Direct feed consumption measurements were not made but visual observations were made to ensure that elvers ate all the feed presented. The inefficiency of feeding by eels meant some of the mash diet was lost into the water column (Gonçalves et al., 1989).

At the beginning and end of experiment, samples of elvers were killed with benzocaine for chemical analyses. For an initial group, 7 elvers were randomly selected from stock tanks, killed and frozen in liquid nitrogen and stored at -80 °C until analysis. After the termination of growth trial, 5 fish from each carboy were killed and 3 fish per carboy were used for the whole body chemical composition.

Diets and dry whole body homogenates were analysed for crude protein (Kjeldahl, selenium catalyst; %N×6.25). Gross energy in diets was analysed by a bomb calorimeter (Gallenkamp Autobomb, calibrated with benzoic acid). Crude fat in diets and dry whole body homogenates were analysed according to the method of Bligh and Dyer (1959). Dry matter (g/kg DM) and ash in diets were

analysed using standard methods (AOAC, 1995).

The following parameters were used to evaluate elver growth performance; Weight gain as WG = (final total tank weight -initial total tank weight); Specific growth rate as SGR (%/day)= [(ln final weight-ln initial weight)×100]/d; Feed efficiency ratio as FER = total weight gain (g)/total feed consumption (g DM); Protein efficiency ratio as PER (%)= [gain in weight (g)/protein intake (g)]×100 and Productive protein value as PPV (%)= [protein retained (g)/protein intake (g)]×100.

3.2.4. Statistical analyses

Data are presented as mean ± S.E.M throughout the text. All the data were subjected to one-factor ANOVA. Prior to ANOVA, assumptions of normality and homogeneity of variances were assessed by Shapiro-Wilk (Zar, 1996) and Cochran's test (Underwood, 1981). When a significant difference was detected by ANOVA, all means were compared with Tukey-Kramer HSD multiple comparison test. All the statistical tests were conducted using JMP version 3.0 statistical software (SAS Institute). Significance was accepted at probabilities of 0.05 or less.

3.3. Results

The highest growth response was obtained with treatment P45 followed by P35, P55 and P25. Total weight gain was significantly higher for the treatment P45 than P25 and P55. However, there was no significant difference between treatments P45 and P35 or P25 and P55. The mortality rate was low in the experiment and there was no significant difference within or between the paired iso-energetic dietary treatments (Table 3.2). The SGR (%/d) value for treatment P45 was significantly higher than that of treatments P55 and P25 but similar to P35 (Table 3.2).

It appeared that feed efficiency ratio (FER), although not significantly, increased with increasing crude protein content in low energy diets (treatments

P25 and P35). However, 10% increase in dietary protein content (from 45 to 55%) in high energy diets resulted in lower FER for treatment P55 but this was also not significantly different from that of treatment P45. The PER (%) was higher in low protein:low energy diets (treatments P25 and P35) than that of high protein:high energy diets (treatments P45 and P55). Although the value for P45 was lower than that of P25 and P35, there was no significant difference between these treatments. The lowest PER (%) value was obtained by the treatment P55 and it was significantly lower than that of the other treatments. The PPV (%) values for treatments followed a similar trend to PER (%) in this experiment. The lowest PPV (%) was obtained by the treatment P55 and it was significantly different from that of the other three treatments (Table 3.2). However, there was no significant relationship among treatments P25, P35 and P45.

Proportional increase in dietary crude protein content in paired isoenergetic diets did not significantly change the whole body protein content of the Australian short-finned eel. However, small increase in whole body protein content with increasing dietary crude protein in each group was detected (Table 3.3). The whole body dry matter content was significantly decreased with dietary treatments regardless of dietary crude protein and energy contents of the treatments. The whole body crude lipid contents, however, significantly increased with lower dietary protein in each energy level (Table 3.3).

3.4. Discussion

This study showed that the diets P35 and P45 produced the best growth performance in juvenile Australian short-finned eel. The data also suggested that the higher protein diet (both P35 and P45 had similar P:E ratio) was better than the P35 % protein diet. Although short-finned eel required a dietary protein to energy ratio of 20.6 (g.CP/GE MJ) for a maximum weight gain, it must be stressed that a limited range of treatments was utilised in the present study. Maximum protein growth occur over a narrow range of dietary protein:energy ratios and it is preferred to have wide range of treatments in experiments investigating the protein sparing effects of non-protein energy yielding substrates with fish species (Boorman, 1980; Lanari et al., 1995).

Table 3.1

Formulation (g/kg diet) and chemical composition of the experimental diets.

	Diets			
	P25	P35	P45	P55
Ingredients				
Fish meal	385.0	539.0	692.0	846.0
Fish oil	176.0	113.0	81.4	40.0
Dextrin	135.0	115.0	89.0	35.0
Bentonite	232.8	158.8	60.0	10.0
α-cellulose	13.0	16.0	19.4	10.8
CMC	40.0	40.0	40.0	40.0
Minerals[1]	12.5	12.5	12.5	12.5
Vitamins[2]	5.0	5.0	5.0	5.0
Stay-C[3]	0.5	0.5	0.5	0.5
B.H.A	0.2	0.2	0.2	0.2
Chemical composition (g/kg DM)				
Moisture	71±0.7	73±2.1	76±3.5	78±2.8
Crude protein	264±0.7	368±0.7	464±0.9	583±1
Crude fat	227±9.9	187±2.1	160±1.4	139±14.1
Ash	284±0.8	245±4.8	182±1.3	164±1.6
GE(MJ/kg)	18.6±2.12	19.1±1.53	22.5±0.25	22.1±0.22
P:E ratio (g.CP/MJ)	14.17	19.24	20.57	26.39

[1]Mineral mixture (g/kg food): According to De la Higuera et al. (1989): CaH_2PO_4; 3.424, $CaCO_3$; 3.265, KH_2PO_4; 2.384; KCl; 0.24, NaCl; 1.442, $MnSO_4.H_2O$; 0.089, $FeSO_4.7H_2O$; 0.36, $MgSO_4$; 1.201, KI; 0.0046, $CuSO_4.5H_2O$; 0.012, $ZnSO_4.7H_2O$; 0.06, $CoSO_4$; 0.007, (Na_2MoO_4); 0.002, Na_2SeO_3; 0.005, $AlSO_4.18H_2O$; 0.004.

[2]Vitamin mixture (g/kg food): According to De la Higuera et al., (1989): calcium pantothenate; 0.13, thiamine; 0.044, riboflavin; 0.109, pyridoxine; 0.033, inositol; 0.874, biotin; 0.001, folic acid; 0.011, choline chloride; 2.623, nicotinic acid; 0.219, cyanocobalamin; 0.002, ascorbic acid; 0.874, retinol; 0.044, menadione; 0.022, α-tocopherol; 0.007, cholecalciferol; 0.009. Individual ingredients were supplied by Sigma-Aldrich Pty.Ltd. and ICN Biochemicals Pty.Ltd. Australia.

[3] Stay-C (L-Ascorbyl-2-polyphosphate) supplied by Hoffman La Roche, Basil, Switzerland

Table 3.2

Effects of dietary increasing crude protein with constant gross energy ratios on growth and feed utilisation by the eel, *A.australis australis*

Parameter	Diet				*P*
	P25	P35	P45	P55	
Total initial weight (g)	55.23±0.06	55.31±0.2	54.91±0.5	55.22±0.2	ns
Total final weight (g)	73.37±5.58^{a}	86.74±3.20^{ab}	93.66±4.31^{b}	81.40±9.44^{ab}	0.0199
Total weight gain (g)	18.16±5.60^{a}	31.44±3.32^{ab}	38.80±4.26^{b}	26.18±9.28^{ab}	0.0176
SGR (%/d)	0.64±0.09^{a}	0.83±0.11^{ab}	1.06±0.12^{b}	0.73±0.08^{a}	0.0065
FER[1]	0.08±0.03	0.14±0.01	0.15±0.03	0.12±0.04	ns
PER (%)	42.40±7.18^{b}	42.52±5.58^{b}	41.83±5.07^{b}	22.94±3.18^{a}	0.0051
PPV (%)	8.31±1.31^{b}	8.24±1.39^{b}	7.83±0.89^{b}	4.87±0.76^{a}	0.0154
Survival (%)	91.12±1.91	87.84±6.92	91.12±1.91	94.35±3.81	ns

Each value is the mean (±S.E.M) of triplicate tanks (n=3). Means in the same row with different superscripts are significantly different (Tukey-Kramer HSD, P<0.05).

[1] 10 % of the total feed presented was assumed to be lost in to water column (see section *3.2.3* for more details).

Table 3.3

Effects of increasing dietary crude protein at two constant gross energy levels on whole body chemical composition by the eel, *A.australis australis*

Parameter	Diets				*P*
	P25	P35	P45	P55	
Dry matter (%)	32.34±0.98	30.26±1.58	31.61±1.50	30.30±0.77	ns
Crude protein (% DM)	51.00±1.90	55.54±2.84	54.28±2.35	56.91±2.42	ns
Crude lipid (% DM)	39.38±3.72^{b}	30.50±3.98^{a}	38.24±4.01^{b}	30.73±1.17^{a}	0.0219

Each value is the mean (±S.E.M) of triplicate tanks (n=3). Means in the same row with different superscripts are significantly different (P<0.05).

Initial group (mean±sd; n=7): 24.62±4.41 % dry matter; 59.63±4.81 % crude protein; 25.04±12.42 % crude lipid.

It was evident that the 10% crude protein increase in diets with high gross energy levels (P45 and P55) significantly decreased the weight gain and the growth efficiencies as opposed to to same level of increment in diets with lower gross energy levels. This has indicated that the majority of the protein in diet P55 might have been used to satisfy the energy requirements for maintenance and lower PER and PPV values obtained on this diet. Similar findings have been reported for the European eel (Degani et al., 1986; Dosoretz and Degani, 1987; García-Gallego et al., 1991;1992;1995; Hidalgo et al., 1992; Sanz et al., 1993). Although the utilisation of proteins for basal energy metabolism is a well established phenomenon (Cho and Kaushik, 1985), many researchers have been able to demonstrate the substantial reduction in the oxidation of protein to satisfy the energy needs of several fish species and thus improving the utilisation of protein for growth with inclusion of non-protein energy yielding macronutrients namely lipids and carbohydrates (Ogino et al., 1976; Page and Andrews, 1973; Daniels and Robinson, 1986; Davies, 1989; Kaushik and Médale, 1994; Einen and Roem; 1997). However, protein-sparing effect of dietary energy seems to operate throughout the range of nitrogen intakes and the interaction between protein and energy presents a level of complexity that a full quantitative description, integrating all aspects, has not been attempted (Boorman, 1980). Fuller et al. (1973) showed continuous responses in nitrogen retention in rats fed in such a way as to vary protein intake, protein quality and energy (starch) to increasing energy intake at all nitrogen intakes. However, at lower non-protein energy intake nitrogen retention tended to a higher limit with proteins of poor quality than with proteins of good quality (Fuller et al., 1973).

Protein and lipid are utilised more effectively than carbohydrates as energy sources by most carnivorous fish species (Cho and Kaushik, 1985; Ohta and Watanabe, 1996). Although eels are considered carnivourus (Lecomte-Finiger, 1983), nutritional studies with eels consistently reported (Gallegher and Matthews, 1987; De la Higuera et al., 1989; García-Gallego et al., 1991;1992;1995; Hidalgo et al., 1992; Sanz et al., 1993) that increased highly digestible carbohydrate content in diets had a beneficial effect on the growth and protein utilisation by the eel up to a level of 38 % of total dietary energy, the level which is much higher than the recommended levels for many of marine and cold water cultured fish species (Cowey et al., 1975; Hardy, 1991; Helland et al., 1991; Shimeno, 1991; Wilson,

1994). However, the size of eels seems to be the determining factor for the beneficial effects of carbohydrates in their diets since younger eels may have a higher protein requirement and reduced tendency towards fattening (Sanz et al., 1993). Although investigation of dietary fat to carbohydrate ratio was not the part of the present study, juvenile eels appeared to favour lipid to carbohydrate ratio around 0.9 for a maximum growth when fed diet P45 (45% dietary protein at 22.5 MJ/kg GE level). However, diet P 35 with a lower GE and increased lipid and carbohydrate kept around the same ratio to that of diet P45, produced almost similar weight gain. Previously Japanese eel was shown to grow well with isoenergetic diets containing 60 to 210 g dextrin per kg DM (Nose and Arai, 1971) and equal amounts of dietary fat. European eels have been subjected to similar investigations (Degani, 1987; Degani and Viola, 1987; García-Gallego et al., 1995) and shown to utilise higher levels of dietary carbohydrate (saccharose, wheat flour and corn starch) for a rapid growth. However, the size of the eels used in these investigations was larger (40 g) than the sizes used in the present study and for the Japanese eel (Nose and Arai, 1971) and suggested the changings of metabolic patterns in the life cycle of this species (Lecomte-Finiger, 1983). Degani (1987) showed that European eels raised on a diet containing 300 g saccharose and 460 g protein per kg DM grew more rapidly than others given a diet with 100 g saccharose and 610 g protein per kg diet (DM). Moreover, the possibility of increasing carbohydrate levels up to 400 g/kg DM of a diet with a reduction of protein content to 250 g/kg DM was shown with larger (30-40 g mean average wet weight) European eels (Hidalgo et al., 1993; Sanz et al., 1993).

The juvenile eels in this study gained less weight when fed P25 diet compared to P35 diet at a constant gross energy suggesting, although feed efficiency ratios (FER) were not significantly different, this reduction in growth was probably due to decreased consumption of the higher energy diet. Thus, protein intake was suboptimal at 25 % protein level (Page and Andrews, 1973; Cho et al., 1976; Marais and Kissil, 1979; Daniels and Robinson, 1986; Davies, 1989; Tibaldi et al., 1996). However, dietary protein utilisation for growth on P25 diet, calculated as PER and PPV was almost similar or slightly better than on P35 diet. Degani and Viola (1987) demonstrated that the reduction of the protein:energy ratio from 22.59 to 13.50 g CP/MJ GE (achieved by increasing carbohydrates) improved the PER and PPV of

proteins used, although the optimum growth was attained with an intermediate protein:energy ratio of 18.42 g CP/MJ GE. García-Gallego et al. (1995) also proposed a further reductions in protein:energy ratio to 10.58 g CP/MJ GE without loosing the beneficial effects of non-protein energy substrates on PER and PPV with a medium sized European eels. This may indicate the higher ability of bigger eel's (30-40 g) using dietary carbohydrates as a precursor for fat deposition. Once the protein requirements for growth have been met, it could be more profitable to obtain fat from fat and/or carbohydrate than to obtain it from protein and this has been shown for other fish species (Lee and Putnam, 1973, Reinitz and Hitzel, 1980; Davies, 1989).

Apart from the quality of diets (i.e. the type of ingredients used or the ratio of certain nutrients in diets), the size and the feeding behaviour of particular species determines the feeding efficiency. The poor FER and PPV values obtained in this study confirms the findings reported by other researchers about feeding inefficiency of eels compared with other important farmed finfish species (Gonçalves et al., 1989; García-Gallego et al., 1991;1995; Hidalgo et al., 1993; Sanz et al., 1993). García-Gallego et al. (1991) found that, in the same size of eels and trout (around 45 g average weight), trout were better feed converters than the European eels judged by approximately 30-40 % lower FCR (Food Conversion Ratio) and PPV values in eels. However, the diet with low protein and high carbohydrate (50 % on DM basis) resulted in these parameters in favour of the European eel (García-Gallego et al., 1991). With much smaller size of eels (1.7 to 5.2 g average weight), several authors demonstrated that feed efficiency was severely compromised (Degani and Viola, 1987; Gonçalves et al., 1989). In line with our findings this was perhaps due to the feeding behaviour of small eels when fed with moist mash diets most of which is lost to the water column (Gonçalves et al., 1991).

Increasing protein levels at two different dietary gross energy levels significantly changed the whole body crude lipid concentration in the juvenile Australian short-finned eel. Dry matter and crude protein concentrations did not significantly change but a slight increase in whole body crude protein concentration with 10% dietary crude protein increment at each energy level was evident. This indicated that more dietary protein was being catabolised for energy needs at the higher protein levels

(Cho et al., 1976). Eels can be considered as a fatty animal and its growth implies the storage of a great amount of energetic reserves for the long migratory journey (Hidalgo et al., 1993). Such a compulsory energy accumulation involves a higher requirement of dietary non-protein energy yielding substrates (Sanz et al., 1993).

The present study showed a protein sparing effect of lipids and carbohydrates in the diets of short-finned eel. Further studies investigating specifically the effects of wide range of dietary carbohydrate to lipid ratios at different protein levels on growth and growth efficiencies may help to improve the feed formulation in Australia and limit the impact of intensive recirculating eel farming effluents on environment. Hormonal and biochemical aspects of feeding biology of this species should also be assessed in order to identify the special nutritional features of eels.

CHAPTER FOUR

Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level[1]

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4.1. Introduction

The main end-product of protein metabolism in teleosts is ammonia and a significant proportion of nitrogenous waste is also excreted as urea (Wood, 1993). Consequently, measurements of ammonia and urea excretion have been used as indicators of the effects of various environmental and nutritional factors on protein metabolism and can give an insight into the nitrogen balance of fish (Rychly and Marina, 1977; Jobling, 1981; Beamish and Thomas, 1984; Perera et al., 1995). Therefore, quantification of ammonia- and urea-nitrogen excretion for fish species in relation to nutrition is important for intensive fish culture operations because protein metabolism partly defines the success of a particular nutritional regimen (Dosdat et al., 1995; Gélineau et al., 1998).

The rate of ammonia excretion increases rapidly in response to feed intake (Savitz, 1971; Brett and Zala, 1975; Jobling, 1981; Ballestrazzi et al., 1994) and the majority of the nitrogen excreted is derived from deamination of amino acids from dietary proteins (Wood, 1993; Brunty et al., 1997). Excretion peaks some hours after feed intake and is mainly dependent upon nitrogen intake, temperature and fish species (Lied and Braaten, 1984; Ramnarine et al., 1987; Kaushik and Cowey, 1990). Dosdat et al. (1996) used the same diet to show that ammonia excretion patterns were related to nitrogen intake in three species of marine fish and indicated no inter-species difference. Conversely urea-nitrogen excretion rates were species specific in turbot and gilthead sea bream (Dosdat et al., 1996). Although in early studies urea-nitrogen excretion was not found to correlate with nitrogen intake in the same way as ammonia-nitrogen excretion (Brett and Zala, 1975), several authors have now demonstrated a linear relationship in flatfish (Kikuchi et al., 1991; Carter et al., 1998; Verbeeten et al., 1999) and eel (Knights, 1985). The mechanism behind this is not clear but the adaptive significance of urea synthesis in some teleosts appears to be ammonia detoxification during times when ammonia can not be freely excreted into the environment, such as a high environmental ammonia concentration (Walsh, 1998).

Studies on the relationship between dietary crude protein and ammonia- and

urea-nitrogen excretion in eels, are limited. However, the effects of dietary protein to energy ratios, feeding frequency and ration size on nitrogenous excretion by several eel species have been reported (Gallagher and Matthews,1987; Poxton and Lloyd, 1989; Owen et al., 1998). This study aimed at demonstrating the effect of increasing dietary crude protein content on ammonia- and urea-nitrogen excretion in juvenile Australian short-finned eel kept in a recirculating culture system in which accumulation of nitrogenous excretory products can cause deterioration of water quality. Due to demand for eels, mainly in Europe and Japan, the aquaculture of this species is of interest in Australia (Skehan and De Silva, 1998). However, there is little information available on its nutritional requirements, feed utilisation or ammonia- and urea-nitrogen excretion. Therefore, quantification of nitrogenous excretion in relation to dietary protein is of importance in optimising feeding regimes in recirculating systems and provides an opportunity to compare excretion by the Australian short-finned eel with other eel species.

4.2. Materials and methods

4.2.1. Fish and experimental conditions

Wild elvers of the Australian short-finned eel (*Anguilla australis australis*, Richardson) supplied by the Inland Fisheries Commission, Tasmania, were first weaned on to a commercial eel diet (Chinda Enterprise Corp., Taiwan) and kept in 360-l round fibreglass holding tanks until used. The experiment was conducted in a recirculating system which consisted of twelve 19-l carboys. There were three trickle tray biofilteration units for per four carboys in the recirculating system. Twelve elvers (2.33±0.02 g) were randomly allocated to each carboy. During weight measurements the elvers were anaesthetised (80 mg/lt, benzocaine) and blotted dry. Fish were acclimatised to the experimental diets and conditions for one week before the excretion rates were measured (Beamish and Thomas, 1984; Jayaram and Beamish, 1992). Uneaten feed and faeces were siphoned out daily before beginning measurements of excretion. Water temperature, D.O. and pH levels were maintained at 25.0±1 C°, 5.9±0.2 mg O_2/l and 6.77±0.1, respectively. Water exchange (normally it was 1.1 ± 0.1

l/m) was not utilised throughout the experimental sampling period (see below). Photoperiod was 12 h:12 h Light:Dark.

4.2.2. Diets

Four experimental diets were formulated to contain 25, 35, 45 and 55% crude protein on a DM basis (Table 4.1). Diets were formulated to make the pairs P25 and P35 or P45 and P55 isoenergetic. Diets contained fish meal and fish oil from jack mackeral, *Trachurus picturatus* (Gibson's Ltd., Tasmania, Australia), dextrin (Bunge Bioproducts,NSW, Australia) and the other ingredients by Sigma-Aldrich Pty.Ltd.(Australia). L-ascorbyl-2-polyphosphate (Stay-C, Roche Pharmaceuticals, Switzerland) was used with vitamin and mineral mixtures as described by De la Higuera et al. (1989). Diets were prepared by mixing dry ingredients in a food mixer for 25 min followed by fish oil for a further 30 min. Diets were analysed for crude protein (Kjeldahl, selenium catalyst; Nx6.25), crude fat by chloroform and methanol extraction (AOAC, 1990) and energy using a bomb calorimeter (LECO AC 350 calibrated with benzoic acid). Ash contents were determined by burning the test diets at 550 C° in a furnace for 16 h.

4.2.3. Experimental procedure and measurements

Treatments, diets with varying crude protein content, were replicated three times. Fish were fed twice a day, at 0900 h and 1700 h at a feeding rate of 6% BW/d. It was ensured that fish ate all the diet presented in each feeding time. Daily rations were prepared by mixing the experimental diets with water and divided in half. After the morning feed, the second half was kept at - 20 C ° until the afternoon feed.

Sampling of carboys for ammonia and urea-nitrogen was blocked over time so that all diets were sampled concurrently and three 8 h sampling periods (0900-1700 h; 1700-0100 h; 0100-0900 h) were used. During sampling water flow to carboys was turned off and each carboy of each treatment was sampled over one 8 h period in each sampling day. The experiment was conducted over 5

days so that each tank was sampled over each of the three 8 h periods, with a day in between sampling. It was previously shown that ammonia levels remained well below toxic concentrations (Wedemeyer, 1996). Triplicate 10 ml water samples were collected for ammonia and urea measurements at 4 and 8 h in each sampling period by pipetting water samples from the middle of a carboy to provide data every 4 h over a 24 h period. Excretion was calculated from the change in ammonia or urea concentration and the water volume in the carboy.

The concentration of ammonia in samples was determined by the phenol-hypochloride method (Solorzano, 1969). Urea was analysed by the urease method (Elliott, 1976). Total ammonia-nitrogen concentration was calculated using a standard curve prepared from ammonium chloride solution. The difference between ammonia concentration before and after urease treatment was used to calculate urea concentration.

4.2.4. Statistical analyses

Data are presented as means ± S.E.M throughout the text. Peak rates of ammonia-nitrogen excretion following feeding were compared by one-factor ANOVA. When a significant treatment effect was observed, a Tukey-Kramer HSD test was used to compare means. The relationship between daily nitrogenous excretion rates and dietary protein levels, as dietary percentage, was described by linear regression of the form y= a+bx, where y is the excretion rate of ammonia- or urea-nitrogen and x is the dietary protein content (% DM). Regression analysis was also used to describe the relationships between nitrogen intake and nitrogenous excretion rates for individual tanks in each treatment. Significance was accepted at probabilities of 0.05 or less.

4.3. Results

4.3.1 Diurnal ammonia and urea-nitrogen excretion rates

Daily ammonia-nitrogen excretion rates increased 4 h after the morning feed (Figure 4.1). Excretion rates were significantly ($P<0.05$) higher for treatments

P45 and P55 than for P25 and P35. The first peak occurred 4-8 h after the morning feed for all treatments (Figure 4.1). Following the afternoon feed, the excretion rates decreased in all of the treatments except P45 (Figure 4.1). The ammonia-nitrogen excretion rates just before the next morning's feeding, were higher than the rates at 0500 h in each treatment. The peak excretion rates following feeding in treatments P45 and P55 were significantly ($P<0.05$) higher than P25 and P35 (Figure 4.1). However, there was no significant difference between treatments P25 and P35 or between P45 and P55.

Urea-nitrogen excretion accounted for between 30-50% of total ammonia-nitrogen excretion rates at each treatment (Table 4.2). The variability in excretion rates was not as pronounced as ammonia-nitrogen rates over a 24 h sampling period (Figure 4.2) and rates were found to be the highest in sampling periods 8 h following each feeding except the treatment P35. The excretion rates in treatments P25 and P35 were the same at 2100 h and 0100 h, respectively.

4.3.2. The relationships between dietary crude protein and nitrogenous excretion rates

Mean daily ammonia-nitrogen excretion rates tended to increase with increasing dietary crude protein (Table 4.2). The excretion rate in treatment P25 was significantly ($P<0.05$) lower than the other treatments. The highest mean daily excretion rate was 1225±0.14 (mg NH_3-N/kg/d) in treatment P55 and it was similar to the rate obtained in the treatment P45. Ammonia-nitrogen excretion as a percentage of consumed nitrogen (C_N) increased steadily with increasing crude protein levels. However, there was no significant difference between the treatments (Table 4.2). The relationship between dietary crude protein (x, % DM) and ammonia-nitrogen excretion (y, mg NH_3-N/kg/d) was described by $y=0.058+0.022x$ ($n=12$; $r^2=0.88$; $P<0.001$). Urea-nitrogen excretion rates did not differ significantly between the treatments (Table 4.2). Urea-nitrogen excretion rates as percentage of nitrogen intake was significantly ($P<0.05$) higher in treatment P25 than the other treatments. However, there was no significant difference between the treatments P35, P45 and P55 (Table 4.2). The highest daily urea-nitrogen excretion as percentage of the daily total

nitrogen excretion was obtained on P25 (42 ± 2.61 %) and compared to 30.3 ± 3.56 %, 25.8 ± 1.45 % and 23.6 ±1.54 % for P35, P45 and P55, respectively. The relationship between dietary percentage of crude protein (x, % DM) and mean daily urea-nitrogen excretion (y, mg urea-N/g/d) rates was described by $y=0.426+0.0044x$ ($n=12$; $r^2=0.55$; $P<0.01$).

The relationship between the mean daily nitrogen intake (C_N) and nitrogenous excretion rates for ammonia- and urea-nitrogen was linear (Figure 4. 3). Total mean nitrogen (ammonia-nitrogen + urea-nitrogen) excretion as percentage of nitrogen intake decreased with increasing crude protein (Table 4.2). The highest value (84.98 ± 5.09 %) was on the treatment P25 and was significantly ($P<0.05$) higher than the other treatments. Values were 75.39 ± 8.10 %, 71.86 ± 5.36 % and 65.22 ± 5.04 % for treatments P35, P45 and P55, respectively. The proportion of urea-nitrogen to total nitrogen excretion rates in treatments also decreased with increasing dietary crude protein (Table 4.2).

4.4. Discussion

Ammonia excretion rates are directly related to dietary nitrogen and protein intake in teleosts (Rychly, 1980; Beamish and Thomas, 1984; Kaushik and Cowey, 1990). Feeds for some fish species typically have a high protein content that supplies a large proportion of dietary energy and results in high nitrogenous excretion. Increasing the dietary level of non-protein digestible energy increases nitrogen retention by decreasing nitrogen losses (Cho and Kaushik, 1985; Kaushik and Oliva-Téles, 1985; Médele et al., 1995). The increase in ammonia excretion with increasing dietary protein was in agreement with previous findings for eels (Gallagher and Matthews, 1987; Degani and Levanon, 1988). For the isoenergetic diet pairs, the higher excretion rates for treatments P35 and P55 compared with treatments P25 and P45 respectively, are probably explained by the protein-sparing effect of non-protein energy yielding substrates at lower dietary protein (Lied and Braaten, 1984; Jobling, 1994; Rodehutscord and Pfeffer, 1999).

Table 4.1

Formulation (g/kg diet) and chemical composition of the experimental diets.

	Diets			
	P25	P35	P45	P55
Ingredients				
Fish meal	385.0	539.0	692.0	846.0
Fish oil	176.0	113.0	81.4	40.0
Dextrin	135.0	115.0	89.0	35.0
Bentonite	232.8	158.8	60.0	10.0
α-cellulose	13.0	16.0	19.4	10.8
CMC	40.0	40.0	40.0	40.0
Minerals[1]	12.5	12.5	12.5	12.5
Vitamins[2]	5.0	5.0	5.0	5.0
Stay-C[3]	0.5	0.5	0.5	0.5
B.H.A	0.2	0.2	0.2	0.2
Chemical composition (g/kg DM)				
Moisture	71±0.7	73±2.1	76±3.5	78±2.8
Crude protein	264±0.7	368±0.7	464±0.9	583±1
Crude fat	227±9.9	187±2.1	160±1.4	139±14.1
Ash	284±0.8	245±4.8	182±1.3	164±1.6
GE(MJ/kg)	18.6±2.12	19.1±1.53	22.5±0.25	22.1±0.22
P:E ratio (g.CP/MJ GE)	14.17	19.24	20.57	26.39

[1]Mineral mixture (g/kg food): According to De la Higuera et al. (1989): CaH_2PO_4; 3.424, $CaCO_3$; 3.265, KH_2PO_4; 2.384; KCl; 0.24, NaCl; 1.442, $MnSO_4.H_2O$; 0.089, $FeSO_4.7H_2O$; 0.36, $MgSO_4$; 1.201, KI; 0.0046, $CuSO_4.5H_2O$; 0.012, $ZnSO_4.7H_2O$; 0.06, $CoSO_4$; 0.007, (Na_2MoO_4); 0.002, Na_2SeO_3; 0.005, $AlSO_4.18H_2O$; 0.004.

[2]Vitamin mixture (g/kg food): According to De la Higuera et al., (1989): calcium pantothenate; 0.13, thiamine; 0.044, riboflavin; 0.109, pyridoxine; 0.033, inositol; 0.874, biotin; 0.001, folic acid; 0.011, choline chloride; 2.623, nicotinic acid; 0.219, cyanocobalamin; 0.002, ascorbic acid; 0.874, retinol; 0.044, menadione; 0.022, α-tocopherol; 0.007, cholecalciferol; 0.009. Individual ingredients were supplied by Sigma-Aldrich Pty.Ltd. and ICN Biochemicals Pty.Ltd. Australia.

[3] Stay-C (L-Ascorbyl-2-polyphosphate).

Table 4.2

Mean daily rates of nitrogenous excretion by the elvers of *A. australis australis* in relation to dietary protein levels and as a percentage of consumed nitrogen (C_N) [1]

	Diets (% crude protein levels)			
	P25	P35	P45	P55
C_N (consumed nitrogen, mg N/kg/d)	1330±20^{a}	1854±40^{b}	2340±10^{c}	2940±30^{d}
E_I (energy intake, kJ/kg/d)	16520±80^{a}	16960±90^{b}	19990±120^{cd}	19640±260^{d}
Ammonia-nitrogen (mg NH_3-N/kg/d)	572±30^{a}	853±110^{bc}	1079±80^{cd}	1225±140^{d}
Urea-nitrogen (mg Urea-N/kg/d)	559±40^{a}	561±60^{a}	603±110^{a}	693±110^{a}
Total nitrogen (mg N/kg/d)	1131±70^{a}	1415±130^{ab}	1681±110^{bc}	1919±170^{c}
Ammonia-nitrogen (% C_N)	43.00±2.47^{a}	46.03±5.97^{a}	46.10±3.96^{a}	41.65±4.40^{a}
Urea-nitrogen (% C_N)	41.99±2.62^{b}	30.29±3.58^{a}	25.76±1.41^{a}	23.57±1.54^{a}
Total nitrogen (% C_N)	84.98±5.09^{b}	76.32±7.20^{ab}	71.86±5.36^{ab}	65.22±5.04^{a}
Urea-N/ammonia-N+urea-N (% C_N)	37.13±0.23^{c}	21.37±2.24^{b}	15.32±0.25^{a}	12.32±0.86^{a}

[1] Values are means ± S.E.M (n=3) and means in the same row with different superscripts are significantly different (P<0.05).

Table 4.3

Summary of maximum rates of nitrogen excretion by Anguillid eel species under different nutritional regimens

Species	Weight (g)	Temperature (C°)	CP (%)[a]	Ration (% BW/d), feeds per day	Ammonia-nitrogen (mg N/kg/h)	Urea-nitrogen (mg N/kg/h)	Reference
A. rostrata	0.75	22-23	30	satiation,1	17.5	NA	Gallagher and Matthews (1987)
			35		30.0		
			40		42.5		
A. anguilla	<10	25	50	satiation,1	53.0	18.0[b]	Knights (1985)
A. anguilla	16.4	23	45	2.5,2	25.0	NA	Poxton and Lloyd (1989)
A. anguilla	45	25	45	0.3,1	0.9	1.0	Owen et al. (1998)
			1.0,1		6.1	1.3	
A. anguilla	250	17	NA	Unfed	NA	38.2[c]	Masoni and Payan (1974)
						12.7[d]	
A. australis	2.3	25	25	6.0,2	29.0	27.5	Present study
			35		49.0	25.0	
			45		68.0	28.5	
			55		74.0	33.0	

[a] Dietary crude protein (%N x 6.25).

[b] 30 ‰ saltwater.

[c] Freshwater.

[d] Saltwater.

Fig.4.1. Fluctuations of daily ammonia-nitrogen (NH_3-N) excretion by the Australian short-finned eel fed varying dietary protein levels. Values are means ± SEM (n=3) for each treatment. [a] Represents initial mean ammonia-nitrogen values for each treatment.

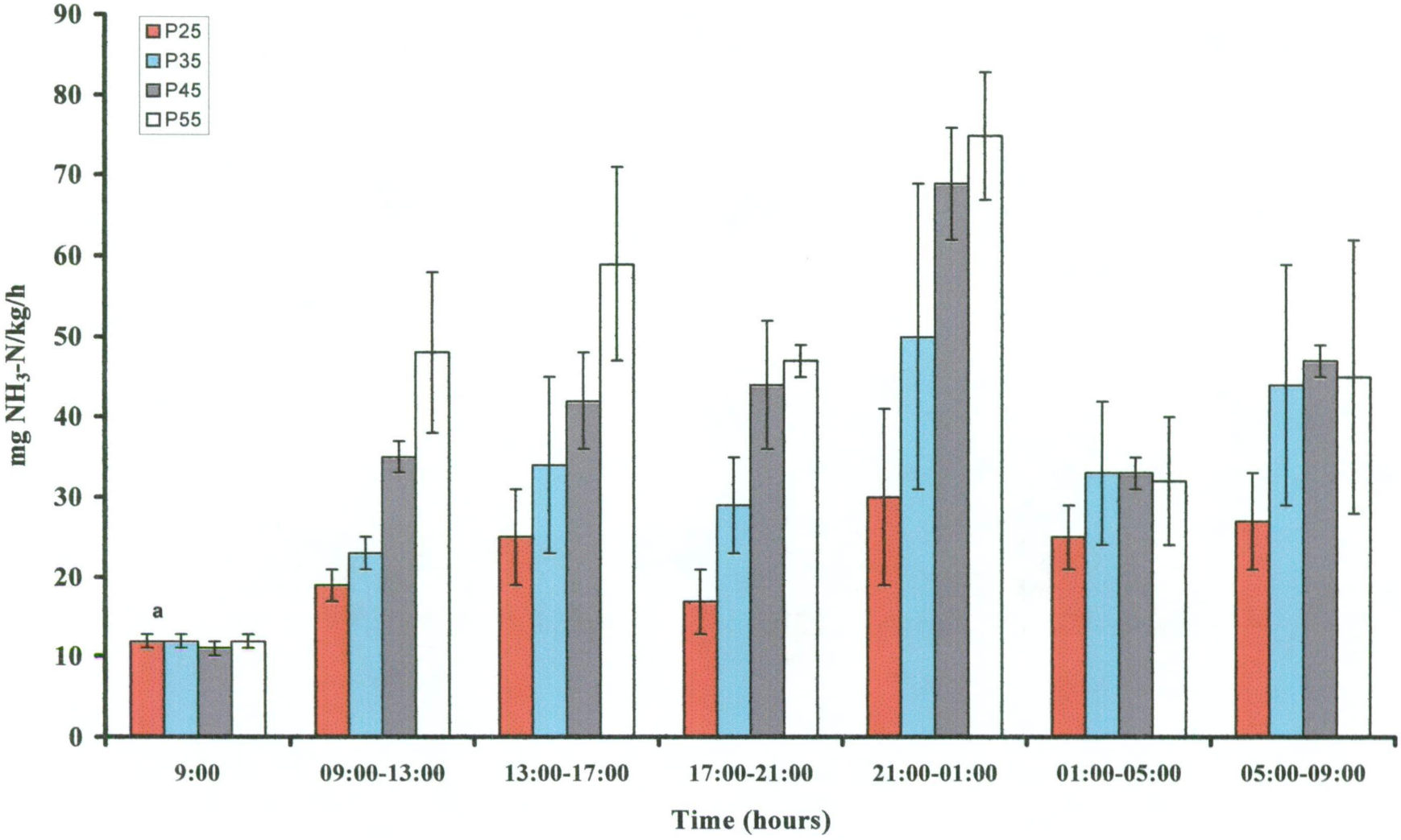

P25
P35
P45
P55
90
80
70
60
50
40
30
20
10
0
mg NH_3-N/kg/h
a
9:00
09:00-13:00
13:00-17:00
17:00-21:00
21:00-01:00
01:00-05:00
05:00-09:00
Time (hours)

Fig.4.2. Fluctuations of daily urea-nitrogen (Urea-N) excretion by the Australian short-finned eel fed varying dietary protein levels. Values are means ± SEM (n=3) for each treatment. **a** represents initial mean urea values for each treatment.

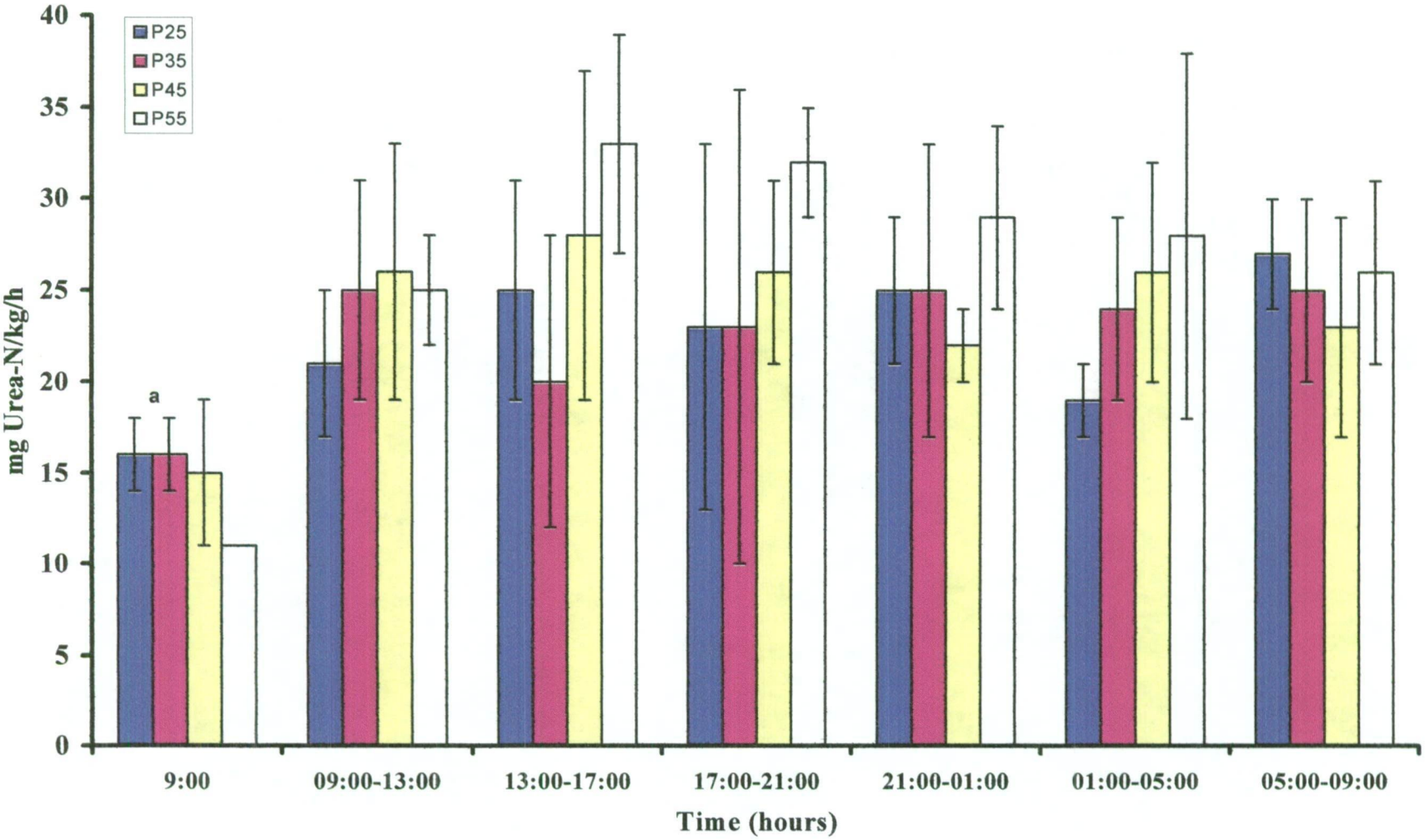
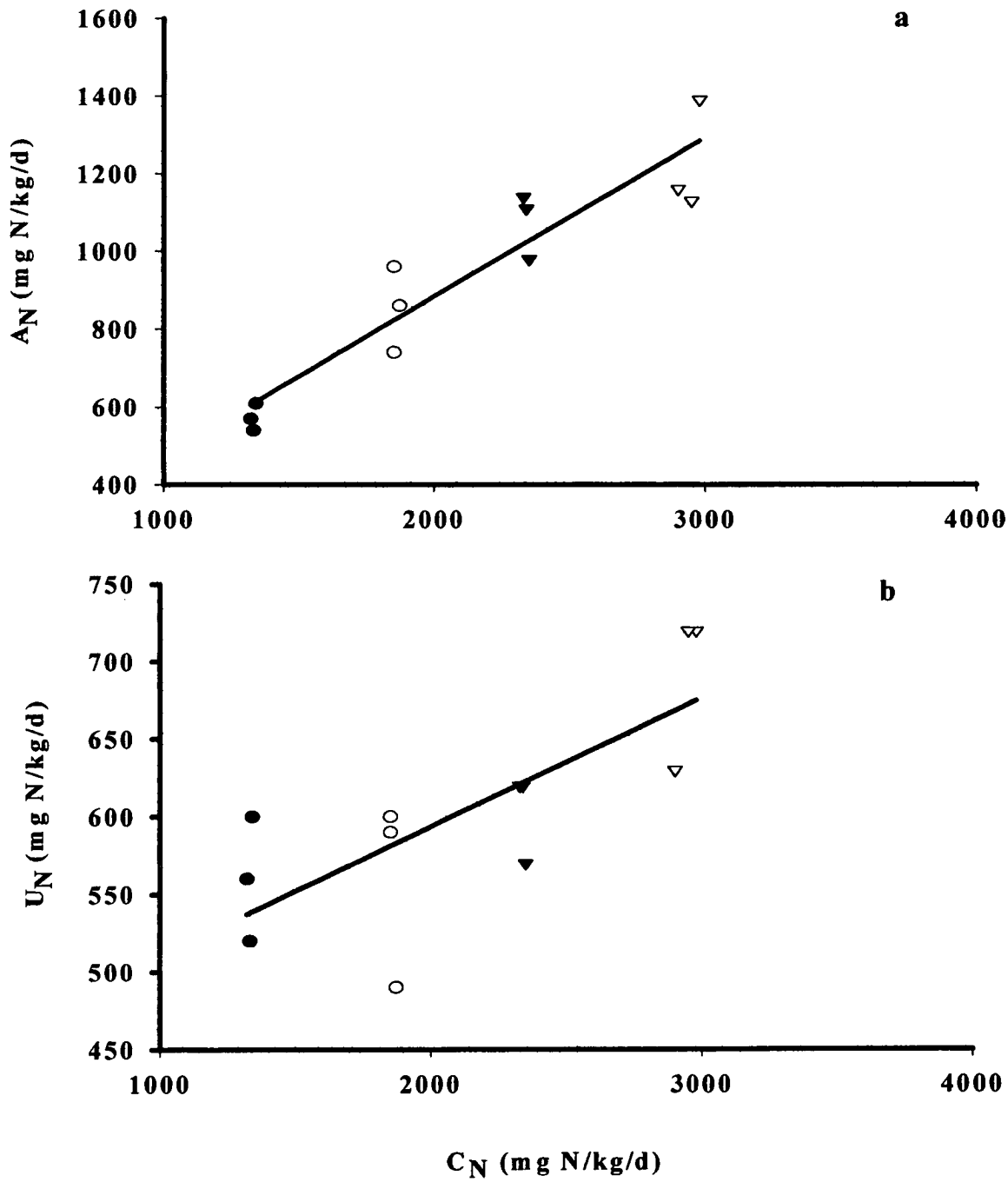

P25
P35
P45
P55
40
35
30
25
20
15
10
5
0
mg Urea-N/kg/h
a
9:00
09:00-13:00
13:00-17:00
17:00-21:00
21:00-01:00
01:00-05:00
05:00-09:00
Time (hours)

Fig.4.3. The relationship between nitrogen intakes and (**a**) ammonia-nitrogen (A_N) (y=0.066+0.41x; n=12; r^2=0.88; P<0.001) (**b**) urea-nitrogen (U_N) (y=0.424+0.085x; n=12; r^2=0.57; P<0.01) for the Australian short-finned eel in individual tanks. ●: P25, O: P35, ▼: P45, ∇: P55.


a
1600
1400
1200
1000
800
600
400
A_N (mg N/kg/d)
1000
2000
3000
4000
b
750
700
650
600
550
500
450
U_N (mg N/kg/d)
1000
2000
3000
4000
C_N (mg N/kg/d)

Gallagher and Matthews (1987) found that when American eels were fed increasing crude protein:energy diets, ammonia excretion increased significantly with increasing crude protein in experimental diets. In their study, the lowest excretion (0.07 ± 0.05 mg NH_3-N/g/h) was obtained at the lowest crude protein:energy ratio diet (16.75 mg/kJ) and the mean increase of 0.05 mg/g/h in ammonia excretion was recorded in between the crude protein:energy ratio levels tested in their experiment (Table 4.3). Degani and Levanon (1988) also reported the increase in ammonia excretion when the protein:energy ratio was maintained. However, overall excretion was lower than found in the present study and measuring excretion under continuous water flow, lower stocking density or the use of larger tanks in their study may have impacted on excretion rates. Biochemical mechanisms of nitrogenous excretion in the Australian short-finned eel, as demonstrated for several fish species (Walsh and Milligan, 1995; McGoogan and Gatlin, 1999), could have related to reduced glutaminase activity on higher dietary non-protein energy diets as ammonia is an end-product of the metabolism of glutamine to glutamate.

The amplitude and time of appearance of peak excretion rates are dependent upon fish size, water temperature and nitrogen intake (Kaushik and Cowey, 1990). There was an immediate increase in ammonia excretion rates following both the morning and afternoon feeds and two visibly distinct peaks occurred 4-8 h after feeding in all the treatments in this study. Clearly two peaks of excretion were related to the two meals fed to the elvers. Large variations in the timing and the magnitude of peak excretion have been reported for different fish (Brett and Zala, 1975; Rychly and Marina, 1977; Ramnarine et al. 1987; Verbeeten et al., 1999), including the European eel (Poxton and Lloyd, 1989; Owen et al., 1998) and the American eel (Gallagher and Matthews, 1987). Brett and Zala (1975) found that following the single morning feed, the excretion rate in sockeye salmon rose sharply to a peak, falling rapidly thereafter in an almost exponential decrease to the early morning base level. Rychly and Marina (1977) demonstrated an increase in blood ammonia levels within 1 h of feeding rainbow trout and proposed that endogenous circadian rhythms in both ammonia excretion and nitrogen metabolism were dependent upon feeding times and nitrogen intake. Feeding twice a day, as used in this study, had a cumulative

effect on ammonia excretion in each treatment and rates after a 24 h period remained higher compared to pre-feeding levels. This was probably due to the increased anticipatory metabolic activity or diel feeding rhythms shown by the elvers. In a study with juvenile Atlantic cod, peak excretion occurred 6.5-27.0 h after feeding depending on ration size and feeding frequency, and declined to pre-feeding levels after 4 days (Ramnarine et al., 1987). This could be both related to the water quality parameters like temperature and pH or diel feeding rhythm of Atlantic cod adapted to a stable feeding regimen. According to Poxton and Lloyd (1989) two meals per day led to the lowest overall production of ammonia by the European eel and that the lowest peak concentration occurred when these feeds were widely spaced out (Table 4.3). Owen et al. (1998) reported that feeding *ad lib* once a day at high ration increased the ammonia-nitrogen excretion more than 10 fold of pre-feeding levels by the European eel and the peak excretion rate occurred 5 h following the start of feeding (Table 4.3). Feeding American eels once a day, also increased the ammonia excretion to a peak 4 h following feeding and returned to pre-feeding level 10 h thereafter (Gallagher and Matthews, 1987).

Fluctuations in daily urea-nitrogen excretion followed the same trend in each dietary treatment, however the overall excretion rates tended to increase with increasing dietary protein (Table 4.2). The level of urea excretion was 30-50% of total-nitrogen excretion rates. Hourly excretion rates were slightly higher 4 h following each feeding. Other workers have found lower and more constant levels (Olson and Fromm, 1971; Brett and Zala, 1975; Cockroft and Du Preez, 1989). For example, Brett and Zala (1975) found that urea-nitrogen excretion by sockeye salmon averaged 2.2±0.2 mg N/kg/h independent of feeding or dark-light switching of the photoperiod control. There is little known about the urea-nitrogen excretion rates in eels. It appears that many previous studies have concentrated on only the ammonia excretion rates in feeding and nutritional studies with eels (Degani et al., 1985; Gallagher and Matthews, 1987; Degani and Levanon, 1988) However, available studies confirmed our results indicating that urea-nitrogen can be an important additional component of nitrogenous excretion in some species, including eels under certain conditions (Masoni and Payan, 1974; Knights, 1985). Masoni and Payan (1974) found significantly

lower branchial clearance values in sea water adapted European eel than freshwater adapted fish indicating a reduced branchial permeability to urea in saltwater (Table 4.3). Knights (1985) also indicated that salinity may have to be taken into account in nitrogenous excretion studies with eels since the author found that urea-nitrogen was higher in freshwater than in seawater (by 120-180% in unfed fish and by 300-330% on one satiation meal of commercial feed; over 50% protein diet on a DM basis) (Table 4.3). This might result from the greater potential for flushing out urea in the higher urine flow rates expected in an hypo-osmotic medium (Eddy, 1981). Highly variable urea-nitrogen excretion and increased urea-nitrogen with increased feed intake were also reported with several other teleosts (Kikuchi, 1995; Harris and Probyn, 1996; Carter et al., 1998; Verbeeten et al., 1999).

The total nitrogenous excretion, expressed as a proportion of consumed nitrogen (C_N), allows an indirect estimate of the proportion of nitrogen retained as growth plus the faecal N. Therefore, the current experiment showed a decrease in nitrogen retention, due to increased urea but not ammonia excretion (as a proportion of nitrogen intake), as dietary protein: energy decreased. This result is difficult to explain since higher nitrogen retention efficiency would be predicted at lower dietary protein intake. Part of the explanation may relate to dietary fat content and the ability of eels to use it (Sanz et al., 1993). At higher dietary fat levels the eels may have had a lower ability to use the fat and therefore used a larger proportion of the protein as an energy source. Because of the changing metabolic patterns in the life cycle of this species (Lecomte-Finiger, 1983), young eels could have a higher protein requirement and reduced tendency towards fattening than the older ones (Sanz et al., 1993). The metabolic nitrogen losses as a % of total nitrogen intake would also depend on the digestable protein intake. This may explain why the absolute increases in ammonia- and urea-N is possible and associated with increased protein diets whilst their relative loss was actually reduced with respect to the total N-intake in the present study. The optimum protein requirement for similar sized Japanese and European eels and larger American eels (8.11±0.07 g) was estimated as between 45 to 50% on DM basis (Nose and Arai, 1972; Degani et al., 1985; Tibbetts et al., 2000). Determination of optimum protein:energy ratio for the

Australian short-finned eel would, therefore, be a useful tool in understanding the pattern of urea excretion observed in the present study. Higher urea loss, as the proportion of total nitrogenous excretion in the treatment P25 and P45 compared to P35 and P55 may also indicate that urea-N excretion in the Australian short-finned eel is more responsive to nutritional variables as it was demonstrated for some flatfish species and lake trout (Jayaram and Beamish, 1992; Dosdat et al., 1995, 1996; Verbeeten et al., 1999).

In conclusion, this study showed that mean daily ammonia-nitrogen excretion rates of juvenile Australian short-finned eel increased when fed increasing dietary crude protein in paired isoenergetic diets. Mean daily urea-nitrogen excretion also tended to increase and accounted for 30-50% of total daily nitrogenous excretion indicating that urea-nitrogen is an important nitrogenous excretory end-product in the Australian short-finned eel. Further studies on the effects of nutritional variables to nitrogenous excretion by the Australian short-finned eel should be used to detail the protein metabolism in this species.

CHAPTER FIVE

The optimum dietary protein:energy requirements of the Australian short-finned eel, *Anguilla australis australis* (Richardson).

5.1. Introduction

The Australian short-finned eel *Anguilla australis australis* (Richardson) is considered to be commercially important (Arai, 1991; Brown et al., 1997; Skehan and De Silva, 1998). Eels, mainly Japanese (*A. japonica*) and European (*A. anguilla*) have been cultured intensively throughout the world (Usui,1974; Arai, 1989; 1991; Degani and Gallagher, 1995; Tibbetts et al., 2000). However, the controlled intensive aquaculture of other eel species including the Australian short-finned eel has been limited, partly due to a lack of understanding of their nutritional requirements.

The determination of the dietary protein requirement is one of the most important aspects to consider during the development of a nutritionally adequate feed. Dietary protein is the most expensive part of commercial feed used for cultured carnivorous fish species and establishing a diet which gives maximum growth at the lowest possible protein level is an obligatory first step in formulation (De la Higuera et al. 1989; Tidwell et al. 1992; Brecka et al. 1995a; Aksnes et al. 1996; Olvera-Novoa et al. 1997). Protein requirement of a given fish species will be mainly influenced by protein quality (availability and balance of amino acids), available energy and environmental temperature (Millikin, 1982; Kim et al. 1991).

Since most eel species are carnivorous, their natural food in the wild mainly consists of protein and fat (Usui, 1974; Lecomte-Finiger, 1983; Dosoretz and Degani, 1987; Degani and Gallagher, 1995; García-Gallego et al. 1995). The dietary protein requirement of eels has been studied for the Japanese (Arai et al. 1971, 1972; Nose and Arai, 1972), European (Spannhof and Kuhne, 1977; Degani et al. 1984, 1985, 1986) and American (Tibbetts et al., 2000) eels. In general, findings suggest optimal levels of 35 to 47% protein for maximum growth rate. However, in defining the optimum level, the suitability of the protein sources (digestibility and amino acid composition) and energy sources (digestibility and availability in relation to inclusion level) need to be taken into account as these may have a significant influence on the outcome.

Fish meal was used as the protein source in this study since the amino acid

profile of the fish meal is preferred to that of other protein sources for teleosts including eels (Arai, 1991). Determining the dietary protein requirement in relation to available energy as well as a proportion of the diet is particularly important since it is the amount of available non-protein energy that determines whether the protein is used for growth efficiently (Cho et al., 1982).

The aim of this experiment was to determine the effect of varying dietary protein level on the growth and growth efficiency of the Australian short-finned eel fed iso-energetic diets and to recommend the optimum dietary digestible crude protein to digestible energy (g.DCP/MJ DE) ratio for maximum growth. In correctly designed nutrient requirement studies, the nutrient fed in graded levels produces a predictable response curve from which the optimum dietary protein can be calculated (Baker, 1986; Mercer et al. 1993; Shearer, 2000). Before making any recommendations on nutrient requirements, best fit for a data set should be attempted using different models since it is evident in nutrient requirement estimations to report different estimations with different models for the same data set (Baker, 1986; Shearer, 2000). Total wet weight gain (g) and protein growth (%) were utilised as physiological responses and modeled both with second order polynomial (quadratic) and 5-SKM (five parameter saturation kinetics model) models in order to estimate the optimum dietary crude protein requirement. The magnitude of estimated values from different models was also compared (Shearer, 2000).

5.2. Materials and methods

5.2.1.Fish and maintenance

Before the trial a total of 300 Australian short-finned eel elvers, randomly selected from the holding tanks, were acclimatised to the experimental system for two weeks. Elvers were fed with the commercial eel diet twice a day during the acclimation period. After the acclimation period all elvers were taken out, pooled and 15 elvers (average wet weight of 1.96±0.2 g) randomly re-allocated to each carboy. Each treatment (dietary protein level) was replicated three times.

Uneaten food and faeces were removed daily from carboys by siphoning. Mean

(± SD) values of water quality parameters were recorded throughout the experiment: 26±0.7 °C water temperature (daily), 6.5±0.1 mg/l DO (twice a week), 6.62±0.3 pH (twice a week), 0.27±0.02 mg/l ammonia-nitrogen (NH_3-N) (twice a week) and 0.016±0.005 mg/l nitrite-nitrogen (NO_2-N) (twice a week).

5.2.2. Diets

Five experimental diets were formulated to contain a crude protein content ranging from 25 to 55% DM (Table 5.1). Fish meal (from jack mackeral, *Trachurus picturatus*, Triabunna, Tasmania, Australia) was used as the only protein source. Fish oil (South American fish oil, Pivot Aquaculture, Cambridge, Tasmania, Australia) and dextrin (Sigma-Aldrich, Sydney, Australia) contents were adjusted to maintain similar dietary energy contents and the total proportions were also adjusted to 100 % by the addition of small amounts (<2.0%) of α- cellulose and bentonite (Sigma-Aldrich, Sydney, Australia). BHA (2[3]-t-butyl-4 hydroxy-anisole, Sigma-Aldrich, Sydney Australia) was used as an anti-oxidant. Stay-C (L-Ascorbyl-2-polyphosphate, Hoffman La Roche, Basil, Switzerland) was used to supplement ascorbic acid in the vitamin premix. Mineral and vitamin premixes were the same as those used by De la Higuera et al. (1989) for European eels.

Diets were prepared by mixing the ingredients in a Hobart mixer. The diets were then manufactured as pellets (1 mm die) using a loboratary pellet mill (Model CL-2, California Pellet Mill Co., U.S.A). All the diets were dried overnight at 37 °C in a fan forced oven. Following drying, diets were individually bagged and stored at 4 C° until used.

5.2.3. Experimental procedure

Elvers were fed twice a day between 0900 and 1000 h and between 1700 and 1800 h on rations equal to 5% BW/d for 91 d. After the morning feed, the other half of the ration was stored at 4° C until the afternoon feed. Following collective weighing (the total weight of fish in each carboy), the ration was adjusted every 2 weeks for the first 4 weeks of experiment and every 3 weeks thereafter (a further 9 weeks). Elvers were anaesthetised (80 mg/l, Benzocaine) during weight and length

measurements and vigorous aeration was used during recovery from the anaesthesia. Elvers were weighed and length measured individually at the beginning and end of the experiment.

Feed consumption was measured once per week. The night before, all tanks were cleaned of the solid organic material and feed consumption (g DM) measured the following day. Corresponding feed consumption values for each replicate tank for a week were then calculated by subtracting the measured uneaten food from the daily rations used in that week.

At the beginning and end of the experiment, samples of elvers were killed with benzocaine for whole body chemical analysis (see below). An initial group of 15 elvers was randomly selected from stock tanks, killed and stored at - 20 °C until analysis. Five elvers from each carboy were killed at the end of the experiment

5.2.4. Chemical analysis

Diets and freeze dried (DYNAVAC®, freeze drier, HST Technology Pty. Ltd., Australia) whole body homogenates were analysed for crude protein (Kjeldahl, selenium catalyst; %N×6.25). Gross energy in diets was analysed by a bomb calorimeter (Gallenkamp Autobomb, calibrated with benzoic acid). Crude fat in diets and dry whole body homogenates were analysed according to the method of Bligh and Dyer (1959). Dry matter (g/kg DM) and ash in diets were analysed using standard methods (AOAC, 1995).

5.2.5. Elver growth performance evaluation

The following parameters were used to evaluate elver growth performance; Feed efficiency ratio as FER = total weight gain (g)/ total feed consumption (g DM); Weight gain as WG = (final total tank weight -initial total tank weight); Specific growth rate as SGR (%/d)= [(ln final weight-ln initial weight)×100]/days; Protein efficiency ratio as PER (%)= [gain in weight (g)/protein intake (g)]×100 and Productive protein value as PPV(%)= [protein retained (g)/protein intake (g)]×100.

5.2.6. Statistical analyses

Data are reported as ± S.E.M throughout the text. Means were compared by one-way ANOVA. Prior to ANOVA assumptions of normality and homogeneity were confirmed for each parameter using Shapiro-Wilk (Zar, 1996) and Cochran's (Underwood, 1981) tests, respectively (JMP Version 3.2.1). When a significant treatment effect was observed a Tukey-Kramer HSD test was used to compare means. Significance was accepted at probabilities of 0.05 or less.

5.2.7. Modeling the optimum dietary protein requirement

In previous nutrient requirement estimation studies with animals, the saturation kinetics models (4 or 5 parameter SKM), broken line model and several non-linear models have all been used with varying success in fitting the results (Robbins et al. 1979; Mercer et al. 1989; Mercer, 1992; Mercer et al. 1993, Shearer, 2000). Dietary protein requirements were estimated as percent digestible crude protein (% DCP) and the optimum digestible dietary protein to digestible energy ratio (g.DCP/MJ DE). The apparent protein digestibility value of a reference diet (Chapter 6) containing fish meal (from jack mackeral, *Trachurus picturatus* Triabunna, Tasmania, Australia) as the only protein source was used in calculation of DCP content in the present study. However, reference dextrin digestibility value of 71% calculated for the channel catfish (NRC, 1993) was used in the calculations of the digestible energy contents of the experimental diets. Fish oil was assumed to be 100% digestible. The estimated digestible dietary protein to digestible dietary energy ratios from two different models were compared. A second order polynomial (quadratic) model using Sigma Plot version 4.0 (Jandel Corporation) and a 5-SKM (five parameter saturation kinetics model) were utilised to estimate the optimum digestible dietary protein to dietary digestible energy ratios. The polynomial regression equation generated for data sets was $y= ax+bx^2+c$ as described by Muller et al. (1996) where y represents response (weight gain (g)), x is the dietary protein level (5 DCP or g.DCP/MJ DE) and a, b and c are empirically derived coefficients. The physiological response was estimated with the equation generated by 5-SKM model (Mercer et al., 1989):

$$r = \frac{b(K_{0.5})^n + R_{max}.I^n + bI^{2n}/(K_s)^n}{(K_{0.5})^n + I^n + I^{2n}/(K_s)^n}$$

where r represents physiological response; I= dietary concentration of the limiting nutrients, b= intercept on y axis, R_{max}=maximum theoretical response, n= apparent kinetic order, $K_{0.5}$= concentration for 1/2 of (R_{max}+b), K_s= inhibition constant.

5.3. Results

5.3.1. Growth performance

Weight gain (g) was significantly higher for the P40 treatment. However, there was no significant difference between treatments P40, P32.5 and P55 (Table 5.2). The lowest weight gain was obtained by the treatment P25, and it was significantly different from the other treatments. The SGR value varied between 0.3 and 0.6%/d among treatments (Table 5.2). The lowest SGR was obtained by the treatment P25 and it was significantly lower than the other treatments. There was no significant difference between the treatments P32.5, P40, P47.5 and P55. There was no mortality throughout the experiment. However, escapees were treated as mortalities but only small numbers of elvers were lost and there were no significant differences between treatments (Table 5.2).

Dietary protein significantly affected the whole body lipid and crude protein content (% DM). However, whole body dry matter was not related to dietary protein (Table 5.3). Increasing dietary crude protein increased the body protein content (Table 5.3). However, there was no significant difference between the values of the treatments P25, P32.5 and P40. High lipid contents in diets also appeared to increase the lipid contents of the whole body in this study (Table 5.3).

The growth and growth efficiency parameters obtained in the present study have been evaluated by both one-way ANOVA and regression models. The purpose of ANOVA was to indicate the statistical differences between treatments and were followed by multiple mean comparisons (Tukey-Kramer HSD test, $P<0.05$) (Table 5.2). Regression analysis, however, was used to estimate a value for the protein

requirement of juvenile Australian short-finned eel from dose response curves based on weight gain (g) using either second order polynomial (quadratic) or 5-SKM (five parameter saturation kinetics) models. Although ANOVA has been used frequently in nutrient requirements from dose response data, its use to analyse this type of data is inappropriate for a number of reasons (Dawkins, 1983). When ANOVA is used, nutrient levels are treated as discrete rather than continuous so that the optimum nutrient level is stated as a range between two input levels (Shearer, 2000). In addition, test power is often low with ANOVA even when variances are small (Shearer, 2000). Therefore, studies evaluating dose response data by regression analysis should have less chance of misinterpreting the requirements providing the best fit is being attempted in regression analysis (Shearer, 2000).

5.3.2. Modeling of the optimum dietary protein requirement

The optimum dietary protein level for the Australian short-finned eel was estimated as 43.0 (±3.5) % DM of the diet when weight gain (g) data were used in the second order polynomial (quadratic) model (Figure 5.1). In terms of dietary g.DCP/MJ DE the optimum dietary protein level was 24.5 (± 1.7) g.DCP/MJ DE (Figure 5.2). However the estimated optimum values appeared to be lower than that of second order polynomial (quadratic) model with 5-SKM. The comparison of the optimum dietary protein requirement estimated with two different models using total weight gain (g) data is shown in Table 5.4 and indicated the estimates were similar being within 6% of each other.

5.4. Discussion

5.4.1. Growth performance

Diets were prepared as isoenergetic with increasing dietary crude protein, dextrin and fish oil as non-protein energy sources in this study. PER and PPV were calculated as indices of the nutritive utilisation of dietary protein (Table 5.2). Even

Table 5.1.

Formulation (g/kg diet) and chemical composition of the experimental diets

	Diets (protein %)				
	P25	P32.5	P40	P47.5	P55
Ingredients					
Fish meal	333.0	432.9	532.8	632.7	732.6
Fish oil	174.6	138.7	99.3	48.5	15.7
Dextrin	227.6	171.8	119.2	100.6	32.4
Bentonite	187.8	180.4	171.9	141.8	143.0
α-cellulose	18.8	18.0	18.6	18.2	18.1
CMC	40.0	40.0	40.0	40.0	40.0
Minerals[1]	12.5	12.5	12.5	12.5	12.5
Vitamins[2]	5.0	5.0	5.0	5.0	5.0
Stay-C[3]	0.5	0.5	0.5	0.5	0.5
B.H.A	0.2	0.2	0.2	0.2	0.2
Chemical composition (g/kg DM)					
Moisture	49.6±0.9	45.2±4.0	54.1±3.2	43.9±2.7	40.8±1.3
Crude protein	248.1±3.0	323.9±3.9	399.0±2.1	476.3±14.3	559.9±10.9
Crude fat	255.2±14.2	203.9±13.6	161.4±4.8	135.8±17.3	94.9±12.2
Ash	242.4±1.1	255.3±12.7	240.9±26.3	244.7±3.6	259.6±0.3
GE (MJ/kg)	17.7±0.1	17.6±0.03	17.4±0.1	17.1±0.1	16.9±0.1
DCP	234.5	304.8	375.1	445.5	515.8
g.DCP/MJ DE	14.47	18.64	22.85	27.03	31.09

[1]Mineral mixture (g/kg food): According to De la Higuera et al. (1989): CaH_2PO_4; 3.424, $CaCO_3$; 3.265, KH_2PO_4; 2.384; KCl; 0.24, NaCl; 1.442, $MnSO_4.H_2O$; 0.089, $FeSO_4.7H_2O$; 0.36, $MgSO_4$; 1.201, KI; 0.0046, $CuSO_4.5H_2O$; 0.012, $ZnSO_4.7H_2O$; 0.06, $CoSO_4$; 0.007, (Na_2MoO_4); 0.002, Na_2SeO_3; 0.005, $AlSO_4.18H_2O$; 0.004.

[2]Vitamin mixture (g/kg food): According to De la Higuera et al (1989): calcium pantothenate; 0.13, thiamine; 0.044, riboflavin; 0.109, pyridoxine; 0.033, inositol; 0.874, biotin; 0.001, folic acid; 0.011, choline chloride; 2.623, nicotinic acid; 0.219, cyanocobalamin; 0.002, ascorbic acid; 0.874, retinol; 0.044, menadione; 0.022, ∝-tocopherol; 0.007, cholecalciferol; 0.009. Individual ingredients were supplied by Sigma-Aldrich pty.ltd. and ICN Biochemicals pty.ltd. Australia.

[3]Stay-C (L-Ascorbyl-2-polyphosphate) was supplied by Hoffman La Roche, Basil, Switzerland.

Table 5.2.

Effect of dietary protein levels on growth and feed utilisation by the short-finned Australian eel *A. australis australis*.

Parameter	Diet					*P*
	P25	P32.5	P40	P47.5	P55	
Total initial weight (g)	28.73±0.46	28.87±0.18	29.02±0.60	29.60±0.20	28.87±0.23	ns
Total final weight (g)	39.19±1.67^{a}	47.12±1.55^{bc}	49.00±0.90^{c}	45.55±0.77^{b}	46.10±0.31^{bc}	<0.0001
Weight gain (g)	10.46±1.72^{a}	18.25±1.63^{bc}	19.98±1.43^{c}	15.95±0.96^{b}	17.21±0.38^{bc}	<0.0001
SGR (%/day)	0.34±0.05^{a}	0.54±0.04^{bcde}	0.58±0.05^{cde}	0.47±0.03^{bcd}	0.51±0.02^{bcde}	0.0002
FER	0.09±0.02^{a}	0.14±0.01^{b}	0.17±0.02^{b}	0.13±0.01^{b}	0.14±0.01^{b}	0.0003
PER (%)	37.71±5.30^{bc}	51.67±3.71^{d}	43.04±3.66^{cd}	29.21±1.39^{ab}	26.84±1.77^{a}	<0.0001
PPV (%)	9.30±0.61^{b}	15.57±1.39^{c}	7.60±0.26^{b}	2.33±0.12^{a}	3.23±0.15^{a}	<0.0001
Survival (%)	93.33±6.65	97.82±3.87	97.82±3.87	100	100	ns

Each value is the mean (± S.E.M.) of triplicate tanks (n=3). Means in the same row with different superscripts are significantly different (P<0.05).

Table 5.3.

Effects of dietary protein levels on whole body chemical composition of short-finned Australian eel *A. australis australis*

Parameter	Diets					*P*
	P25	P32.5	P40	P47.5	P55	
Dry matter (%)	33.14±1.60	32.95±1.82	34.98±5.08	30.81±1.55	30.81±1.65	ns
Crude protein (% DM)	56.73±4.05^{ab}	55.54±4.94^{ab}	54.57±4.90^{a}	59.61±3.73^{b}	64.63±4.66^{c}	<0.0001
Crude lipid (% DM)	36.97±5.98^{b}	35.86±5.87^{b}	36.76±4.48^{b}	29.44±5.24^{a}	27.48±3.85^{a}	<0.0001

Each value is the mean (± S.E.M.) of triplicate tanks (n=15). Means in the same row with different superscripts are significantly different (P<0.05).

Initial group (mean ± sd; n=6): 32.34±2.25% dry matter; 57.31±4.33% crude protein; 32.14±5.86% crude lipid.

Table 5.4

Comparisons of the estimated dietary protein requirement of short-finned Australian eel, *A. australis australis* as DCP (%) and g.DCP/MJ DE with second order polynomial (quadratic) and 5-SKM (five parameter saturation kinetics) models from total weight gain (g) data

Parameter	Model	
	Second order polynomial (quadratic)	5-SKM (five parameter saturation kinetics)
DCP (%)		
Model	$y=2.56x-0.031x^2-33.18$[a]	[b] $r=\frac{-380.3\times(15.7)^{0.6}+480\times I^{0.6}-380.3\times I^{1.2}/(105.4)^{0.6}}{(15.7)^{0.6}+I^{0.6}+I^{1.2}/(105.4)^{0.6}}$
Requirement	43.0 (±3.5)	40.6
g.DCP/MJ DE		
Model	$y=4.18x-0.085x^2-32.07$	$r=\frac{-410.5\times(7.6)^{0.6}+462.4\times I^{0.6}-410.5\times I^{1.2}/(70.8)^{0.6}}{(7.6)^{0.6}+I^{0.6}+I^{1.2}/(70.8)^{0.6}}$
Requirement	24.5 (±1.7)	23.2
Magnitude of difference in estimated values (%)[1]		
DCP (%)	94	
g.DCP/MJ DE	95	

[a] y=response

x=dietary concentration

[b] r=physiological response

I=dietary concentration

[1] Estimated dietary requirement as DCP (%) or g.DCP/MJ DE by 5-SKM/Estimates by second order polynomial (quadratic) (Shearer, 2000)

Table 5.5.

The optimum protein requirements (CP % diet and g.CP/MJGE) of the *Anguillid* eels

Species	Size (initial, g)	Protein sources	Model used	Range studied (% CP)	Requirement		Performance		Reference
					CP (%)	P:E ratio (g CP/MJGE)	Growth rate (%WG/d)	N retention (%)[6]	
A. japonica[2]	3.0	vitamin free casein	NA	0-62	44.5	24.12	1.66	NA	Nose and Arai (1972)
A. anguilla[3]	40.0	white fish meal	NA	36-55	40.6	19.97	0.92	2.1	De la Higuera et al. (1989)
	40.0	herring meal	NA	34-56	44.1	23.01	0.49	0.9	
A. anguilla[4]	1.5	poultry meal	NA	25-55	45.0	21.17	3.05	NA	Degani et al. (1985)
A.rostrata[5]	8.1	herring meal	NA	35-51	47.0	22.00[a]	2.09	2.2	Tibbetts et al. (2000)
A.australis	2.0	jack mackeral meal	5-SKM Second order polynomial	25-55	43.0 (±3.5)[ab] 40.6[ac]	24.5 (±1.7)[ab] 23.2[ac]	1.30	1.6	This study

[2]8 week feeding period.

[3]12 week feeding period.

[4]17 week feeding period.

[5]12 week feeding period.

[6](total retained/total fed) X 100.

[a]on a g.DCP/MJ DE basis.

[b]values esimated by a second order polynomial (quadratic) model.

[c]values estimated by a 5SKM (five parameter saturation kinetics model).

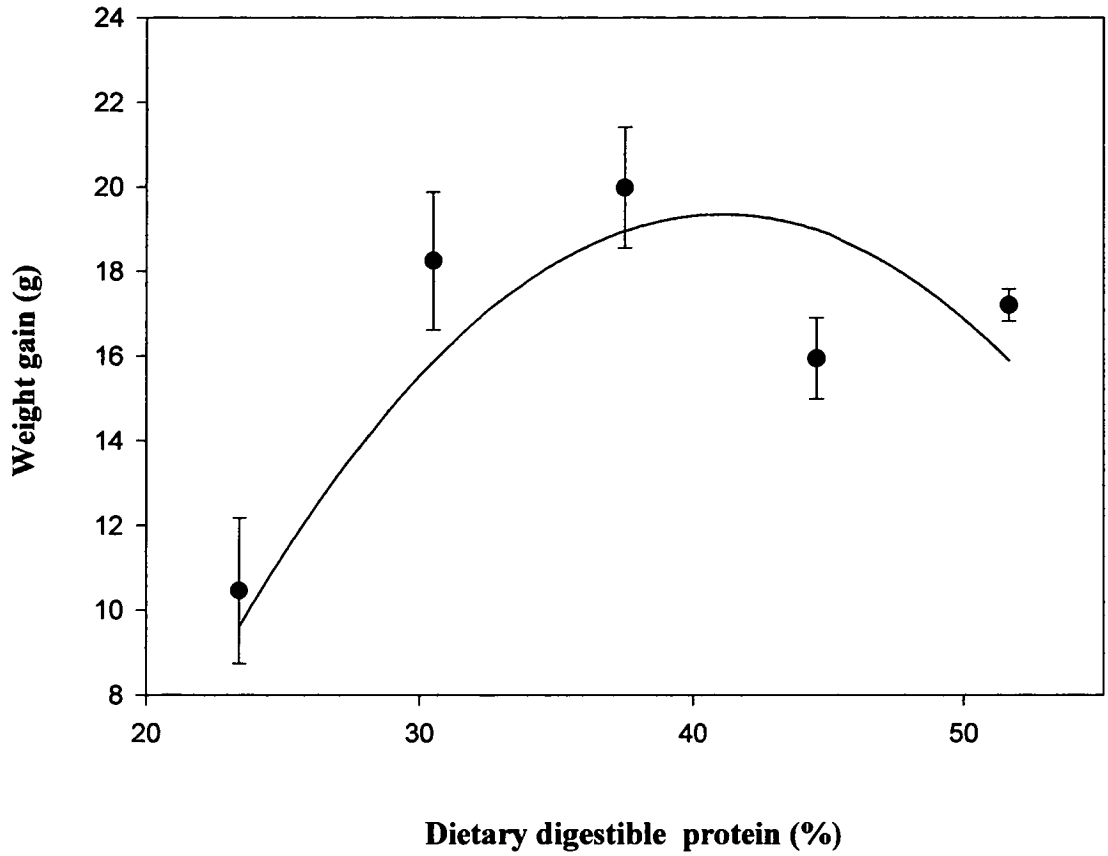


Figure 5.1. The estimation of dietary protein requirements of *A. a. australis* as a dietary digestible percentage using weight gain (g) . Values are ± S.E.M for each treatment ($y=2.56x-0.031x^2-33.18$, $n=5$, $r^2=0.79$, $F=9.2157$, $P=0.0210$).

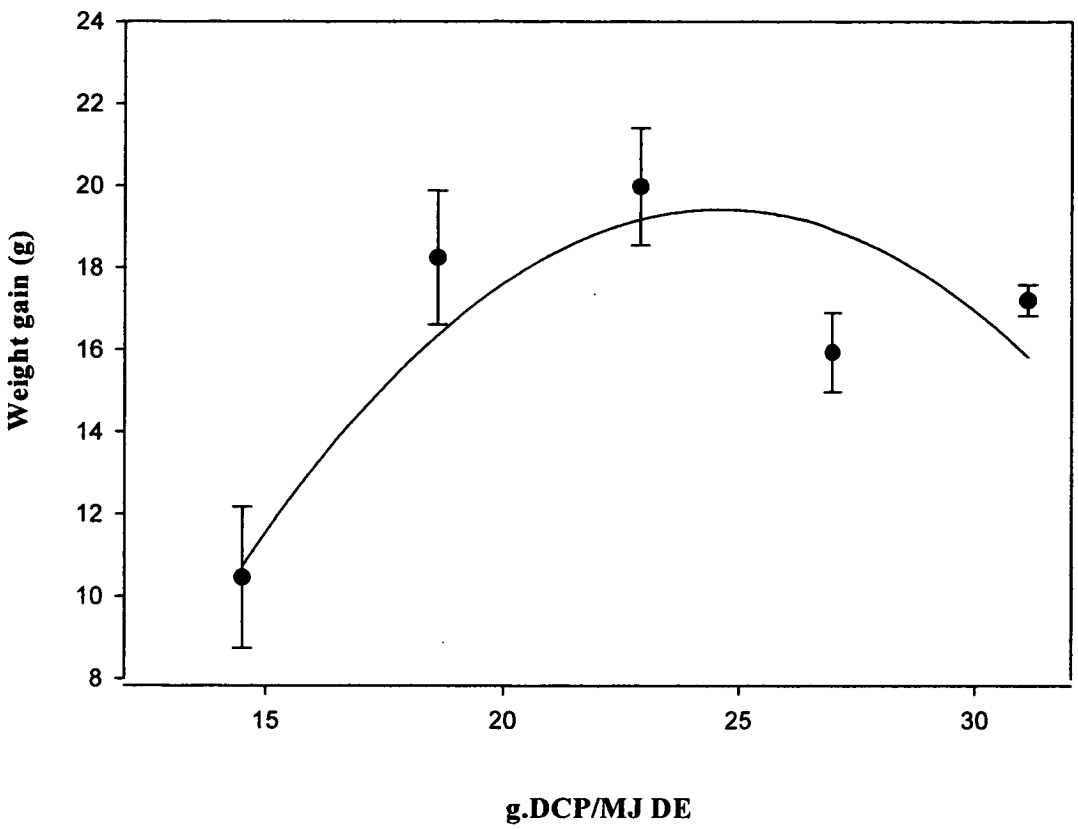


Figure 5.2. Estimated dietary protein requirement of *A.a. australis* in terms of DCP:DE ratios using weight gain (g) increases over the experimental period. Values are ± S.E.M for each treatment ($y=4.18x-0.085x^2-32.07$, $n=5$, $r^2=0.83$, $F=12.0573$, $P=0.012$).

Figure 5.3. The estimation of dietary protein requirements of *A. australis australis* as a dietary digestible percentage using weight gain (g) by 5-SKM (five parameter saturation kinetics model). n=3 for each treatment.

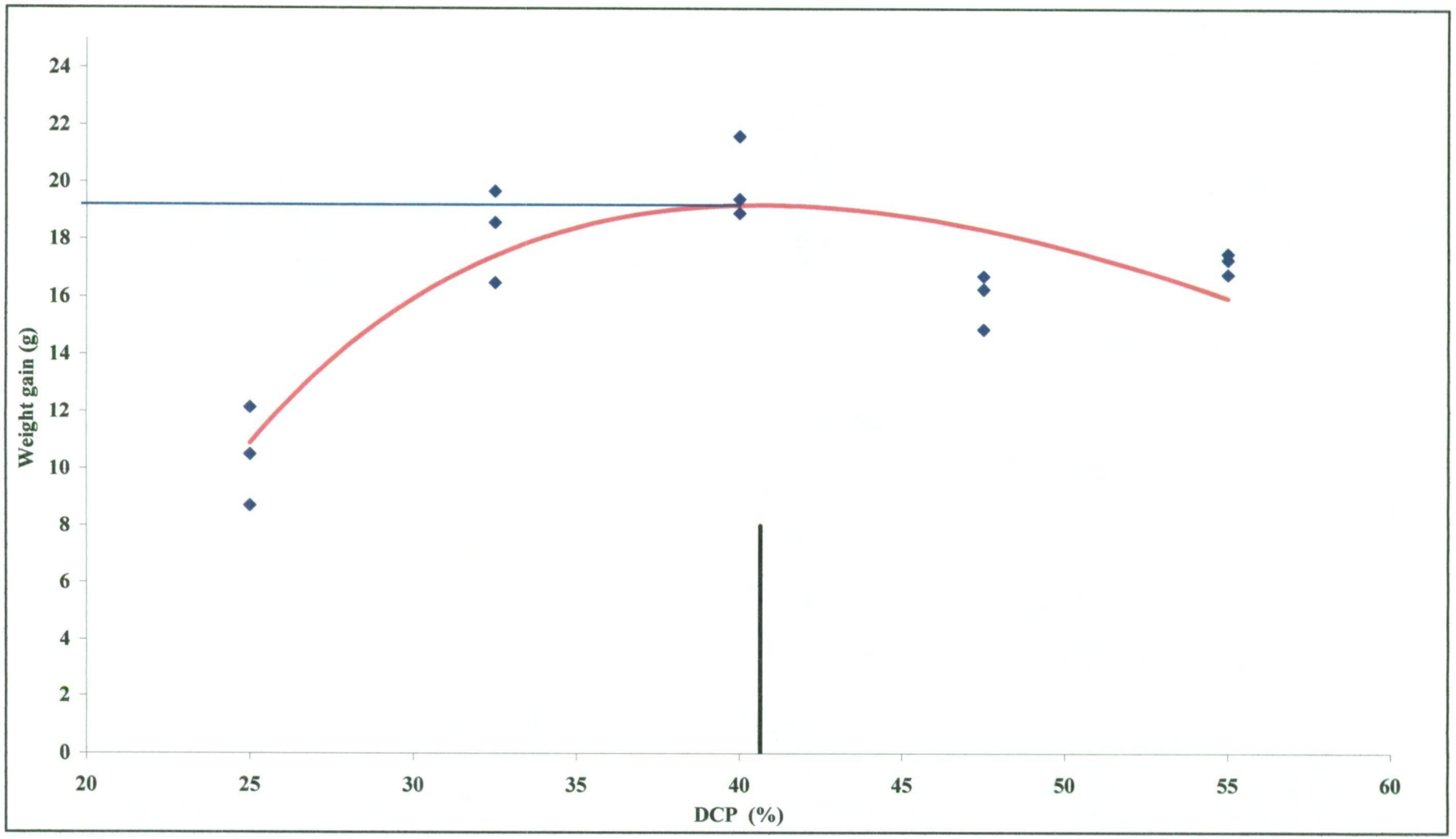
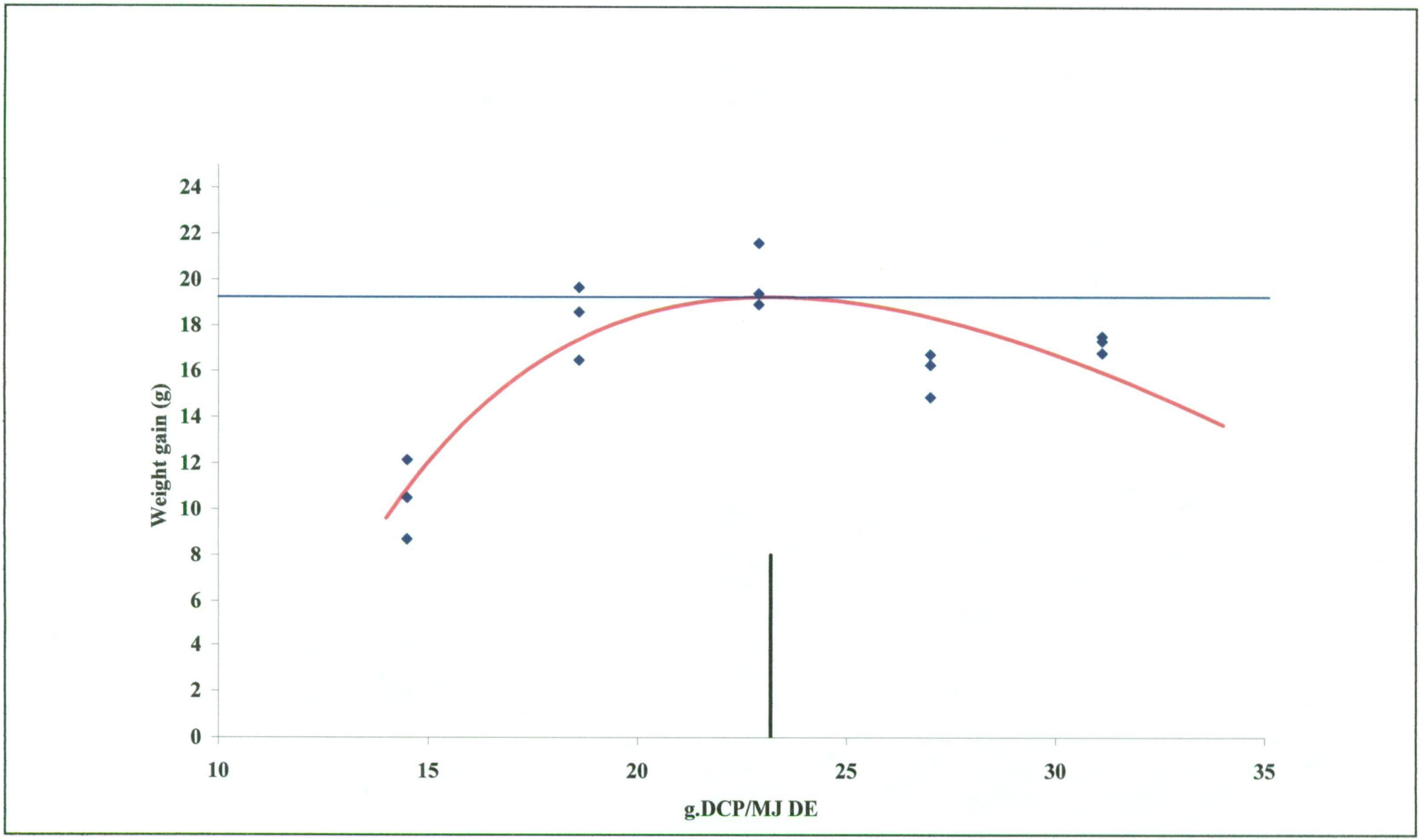

Weight gain (g)
24
22
20
18
16
14
12
10
8
6
4
2
0
20
25
30
35
40
45
50
55
60
DCP (%)

Figure 5.4. The estimation of dietary protein requirement of *A. australis australis* estimated g. DCP/MJ DE using weight gain (g) by 5-SKM (five parameter saturation kinetics) model. n=3 for each treatment.


Weight gain (g)
24
22
20
18
16
14
12
10
8
6
4
2
0
10
15
20
25
30
35
g.DCP/MJ DE

though the values for PPV calculated were much lower than those reported for several other species of cultured fish, they were similar to results found for other eel species (Viola et al., 1984; De la Higuera et al., 1989; Gonçalves et al., 1989; Hidalgo et al., 1993). The specific values tended to increase with increasing levels of dietary carbohydrate (dextrin) and fat indicating the protein sparing effects of non-protein energy yielding substrates in fish feeds. The lowest PER and PPV values obtained by the treatment P55 (the highest protein content diet) indicated that most of the protein in the diet was utilised as an energy source and excreted. It is well known that a high level of fish meal (protein) with low non-protein energy content in the diet increases the oxidation of protein to satisfy the energy needs of the fish and therefore a high excretion of non-feacal nitrogen results (Cho and Kaushik, 1985). The negative effect of higher than optimum protein levels on growth has also been observed in determination of protein requirements of other fish species such as plaice *Pleuronectes platessa* (Cowey et al. 1972), carp, *Cyprinus carpio* L. (Ogino and Saito, 1970), tilapia, *Sarotherodon mossambicus* (Jauncey, 1982), snake head *Channa striata* (Mohanty and Samantaray, 1996) and greenback flounder, *Rhombosolea tapirina* (Bharadwaj, 1997) and an eel *A. rostrata* (Tibbetts et al., 2000). However, this was not observed with the Japanese eel (Nose and Arai, 1972) although Bilio et al. (1979) and Degani (1987) testing the effects of protein level on the European eel growth reported a decrease in weight gain at high protein levels.

The dietary protein saved by the addition of carbohydrates has been reported for many fish species including salmonids (Lee and Putnam, 1973; Kaushik and Oliva-Teles, 1985), plaice (Cowey et al. 1975), catfish (Lovell, 1989) and the European eel (Degani and Viola, 1987; Hidalgo et al. 1993; Sanz et al. 1993). Although this experimentation was not designed to specifically investigate the effects of fat:carbohydrate ratios on growth rates , the results obtained from treatments P32.5 and P40 indicated that fat to carbohydrate ratio of 0.8 (as g/kg and at least up to 10% of the total ingredients of diets) positively affected both the growth rates and the protein utilisation indices of the Australian short-finned elvers. Sanz et al. (1993) reported with the European eel that carbohydrates were similar to lipids in terms of their protein saving ability. Degani and Viola (1987) found that the increase in the percentage of available carbohydrate (wheat meal) reduced the amount of protein or fat used for energy , therefore, the growth rate of the European eels was not reduced.

However, it must be emphasized that the size of the eels used in experiments is an important factor in determination of favourable use of non-protein energy yielding substrates on protein utilisation. In fact, the bigger sizes of eels (35-45 g) in the study of Sanz et al. (1993) and Hidalgo et al. (1993) utilised carbohydrates (when up to 38 % of dietary energy comes from carbohydrates) more efficiently than lipids at producing this sparing action on protein utilisation as opposed to the findings with smaller size of eels in our study (1.96 g mean weight) and the study by Degani and Viola (1987) (4.5 and 5.5 g mean weight elvers). Perhaps, this was due to different nutrient requirements or the greater ability of adult eel's using carbohydrates as precursor for fat deposition (Chan and Woo, 1978; Degani et al. 1986; Hidalgo et al. 1993).

Different dietary protein levels in diets appeared to affect whole body protein and lipid contents of the Australian short-finned eel directly. This was in agreement with studies on other fish species including the Japanese, European and American eels (Nose and Arai, 1972; Page and Andrews, 1973; Millikin, 1982; Degani and Viola, 1987; Tibbetts et al. 2000). It appears that whole body protein was influenced to a greater degree by dietary non-protein energy sources in diets. The higher whole body crude lipid levels in eels in treatment P40 indicated that during growth, elvers accumulated fat. Decreased percentage of protein in the whole body composition in treatment P40 compared to P32.5 also indicated that some part of protein energy in the diet has been used for fat deposition. The similar trend had also been reported for the European and the American eels (Gallagher et al. 1984; Degani et al. 1986).

5.4.2. Dietary protein requirement

The optimal dietary protein level required for maximal growth in farmed fishes is reported to be between 35 and 55% or an equivalent of 45-75% of the gross energy content of the diet should be in the form of protein (Tacon and Cowey, 1985). Because fish may use a proportion of the dietary protein for energy, the optimum dietary protein levels for species are described better as protein:energy ratio preferably on a digestible basis. For eel species, the data available on optimal protein:energy ratios are limited (García-Gallego et al. 1995). This study found that best growth performance for the Australian short-finned eel was obtained from the

diet with the DCP/DE of 22.9 g.DCP/MJ DE for a protein level of 40% DM and an digestible energy level of 17.4 MJ/kg (treatment P40). Degani et al. (1987) reported that for the European eel (*A. anguilla*) the optimum protein:energy ratio is 23.90 g.CP/MJ for a protein level of 45% DM and a gross energy level of 18.79 MJ/kg with carbohydrates as the principal non-protein energy source. A recent study by Tibbetts et al. (2000) recommended 47 % or 22 g DCP/MJ DE for the practical feed formulations of the American eel, *A. rostrata* (Table 5.5). Since there was no significant difference between treatments P40 and P32.5 in terms of growth rates, it appears that the practical protein requirement of the Australian short-finned fingerlings could be lowered down to a minimum 39% DM in a diet or 22 g.DCP/MJ as the optimum DCP/DE ratio. Similarly, Degani and Viola (1987) reported that the optimum Protein:Energy ratio in diets of the European eel could be reduced from 22.59 to 18.42 g.CP/MJ by reducing the protein levels and raising those of the carbohydrates.

Most of the studies with eel species indicated the dietary protein requirements for eels as a percentage of diets (eg. Nose and Arai, 1972; Degani et al. 1985; De la Higuera et al. 1989) (Table 5.5). Nose and Arai (1972) reported that the Japanese eel (*A. japonica*) requires approximately 45% protein in their diet for the maximum growth at 25 C°. The European eel seemed to require between 42.4 (De la Higuera et al. 1989) and 45% CP (Degani et al. 1985) for the maximum growth. However, it is important to note that those requirements were determined by using different protein sources in experimental diets: vitamin-free casein supplemented with L-arginine and L-cystine (Nose and Arai, 1972), chicken meal and chicken meal and fish meal (Degani et al. 1985) and white fish meal and herring meal (De la Higuera et al. 1989). Since free amino acids are not as well utilised as protein bound amino acids (Yamada et al. 1981), protein requirement levels should be determined by using a fixed protein source, fish meal being preferable (De la Higuera et al. 1989; Tibbetts et al., 2000). Different results within or between eel species could also be attributed to differential methodology, non-protein energy sources, feeding regimes, fish age classes and methods for the determination of dietary energy contents (Tacon and Cowey, 1985).

Many fish species have been shown to have a high protein requirement than

endothermic farm animals (Bowen, 1987). The absolute difference in protein requirements of fish species is believed to be due to their differential requirements for energy not protein (NRC, 1983; Tacon and Cowey, 1985; Bowen, 1987). The majority of the protein requirement studies with fish species are based on relative measures and the relative concentration of protein in the diet required for maximum growth does not, on its own, establish a high protein requirement in fishes. Tacon and Cowey (1985) proposed measurements of protein requirements in terms of daily protein intake (milligrams per gram of body weight per day) required to support maximum growth. Although this approach has an advantage that it quantifies protein requirement in absolute terms, considerable variation is introduced in requirement estimates due to differences in physiological age and growth potential of fish species held under different conditions of temperature, photopheriod or some combinations of these variables (Bowen, 1987).

5.4.3. The estimation of optimum protein:energy requirement

The second order polynomial (quadratic) and 5-SKM (five parameter saturation kinetics model) provided the most accurate fit for the estimation of protein requirements of the Australian short-finned elvers using total weight gain (g) increase in this experiment (Table 5.4). The models successfully predicted that the optimum protein requirement of the Australian short-finned fingerlings would be between 39-45 % DM as dietary percentage or between 22.5-26.0 g.DCP/MJ DE ratios in diets (Figure 5.1,2,3,4). One common design problem in nutrient requirement studies is that the determination of sufficiently low and high levels of the nutrient which in return affects the production of typical dose-response relationship (Shearer, 2000). Although the range of nutrient (dietary protein) dose used in this study encompasses the range recommended for most teleosts (Tacon and Cowey, 1985). An ideal experimental design for determination of nutrient requirements would be based on six or seven different doses of the limiting factor with smaller increments (eg. 25, 30, 35, 40 to 55%) and comparing the responses with two different models in order to get more accurate results (Robbins et al. 1979, Shearer, 2000). It is apparent that some previous protein requirement estimations for eels conducted by De la Higuera et al. (1989) and Tibbetts et al. (2000) did not have sufficiently low protein levels to fit a typical dose-response curve. Since 30 and 40%

dietary protein requirements were reported for the European eel using casein and white fish meal as protein sources (Arai, 1986; De la Higuera et al., 1989), it would have been beneficial to include dietary protein level lower than 30% in these studies as sufficiently low level in determination of optimum protein requirements in eels.

The similarity of the estimations between the second order polynomial and 5-SKM models in this study suggests the selection of the appropriate methods and models for statistical analysis. Re-evaluation of the previously published nutrient requirement papers by Shearer (2000) showed that the studies that used broken-line analysis produced estimates averaging approximately 200% of the published values (range 120-500%) (a value of 100% means equal estimates between original and re-evaluation estimate). Therefore, 94 and 95% as magnitude of difference in estimated values from second order polynomial (quadratic) and 5-SKM (five parameter saturation kinetics) models in the present study may also indicate the adequacy of model fit for a dose response data (Shearer, 2000).

In conclusion, this study showed that the Australian short-finned eel required 43.0(±3.5) % dietary protein DM basis or 24.5(±1.7) g.DCP/MJ DE. It was also evident that eels are fatty animals since some protein energy in the treatment P40, which gave the best growth rate, was used for fat deposition. Further studies on bioenergetics or energy budgets with the Australian short-finned eel should provide more detailed knowledge about mechanisms of protein utilisation.

CHAPTER SIX

The apparent crude protein, energy and dry matter digestibility coefficients of selected alternative protein ingredients fed to the Australian short-finned eel, *Anguilla australis australis* (Richardson).

6.1. Introduction

It is well documented that the continuing expansion of aquaculture production necessitates the identification of alternative protein sources to fish meal since fish meal is a major and an expensive component of commercial fish feeds (Robaina et al., 1995; Hardy, 1996; Carter and Hauler, 2000). The quality and suitability of a protein source is mainly dependent on its digestible protein content and amino acid profile (Kaushik and Cowey, 1991; Watanabe et al., 1996; García-Gallego et al., 1998; Gomes et al., 1998). Apart from unbalanced amino acid profiles and endogenous antinutritional factors, the quantity and chemical composition of carbohydrates prevent the use of high levels of plant proteins in fish feeds (Wilson, 1994; Robaina et al., 1995; Refstie, et al., 1998; Mwachireya et al., 1999). Animal by-products may also greatly differ in terms of protein quality resulting from the manufacturing practises used (Johnson et al., 1998). As many of these factors may influence digestibility, it is important to determine the apparent digestibility coefficients using accurate measurement techniques in order to limit the confounding error of a measurement technique on digestibility coefficients (Storebakken et al., 1998).

Techniques to collect faecal material in fish digestibility studies vary including settlement type collectors mechanical collectors, stripping or dissecting the anterior or posterior sections of the intestinal tract, pipetting or siphoning the digesta from the tanks (Choubert et al., 1982; Cho et al., 1982; McGoogan and Reigh, 1996; Allan et al., 1998; Storebakken et al., 1998). Guelph-type settlement faecal collectors are widely used in digestibility studies since they allow the use of smaller sizes of fish and create very little disturbance to faeces or fish during collection compared with pipetting or siphoning the digesta from the bottom of the tank (Hajen et al., 1993; da Silva and Oliva-Teles, 1998). However, overestimation of digestibility coefficients may occur due to nutrient leaching from the faeces in settlement type collectors as opposed to underestimation of digestibility with stripping or dissecting the intestinal tract. Several studies reported digestibility coefficients of balanced diets in the European eel measured with modified Guelph-type settlement collectors (García-Gallego et al., 1995,1998,1999). However only two studies investigated the apparent digestibility coefficients of selected ingredients for eels (Schmitz et al.,

1984; De Silva et al., 2000) and used a specifically designed metabolic chamber or siphoning the digesta out of culture tanks as faecal collection techniques, respectively. Australia has a relatively large variety of non-fish meal protein sources and the nutritional quality of these sources requires investigation prior to the development of balanced diets for feasible farming of the Australian short-finned eel, *Anguilla australis* (Richardson), that is considered as one of the prime candidates for inland aquaculture in Australia (Brown et al., 1997). Traditionally, commercial eel diets contain high levels of prime quality fish meal and research on the possibility of using plant proteins and animal by-products as alternative protein sources to fish meal is limited. The aim of this experiment was to measure the apparent digestibility levels of selected Australian plant protein meals (soybean, canola, corn gluten, lupin and field pea) and animal by-products (blood meal, meat meal, poultry meal). The suitability of a specific modification of the Guelph-type faecal collector, developed for pelagic fish, was also investigated for juvenile eels.

6.2. Materials and Methods

6.2.1. Fish and maintenance

Elvers were supplied by Inland Fisheries Commission, Tasmania. Prior to experimentation, elvers were kept in 380-l stock tanks and weaned on to a commercial diet (Chinda Corp. Taiwan). The digestibility trial was conducted in three modified 19.8-l carboys incorporated into a recirculation system (Engin and Carter, 2001). Three weeks before beginning the experiment, all elvers were transferred to a fifteen-carboy recirculation system and fed the commercial eel diet. Seventy elvers (3.15±0.42 g) were randomly selected from these tanks, weighed and allocated to each carboy of the digestibility system. Elvers were anaesthetised (80 mg/l, Benzocaine).

The experimental system of three tanks did not allow the eight experimental diets and a reference diet to be fed simultaneously in triplicate, the digestibility trial was conducted in 13-d cycles. After a 6-d acclimation period to each diet, faeces were collected for the following 7-d period for each replicate tank of each dietary treatment. Dietary treatments were blocked by tank and randomly allocated over

time so that each of the eight test diets and a reference diet had been fed in triplicate. Elvers were fed at 2.5% BW at 0900 to 1000 h and 1700 to 1800 h each day.

Faeces from each tank was collected using a modified Guelph settlement collector (Cho et al., 1982) attached to each carboy. A 5 mm plastic mesh was firmly attached to the effluent pipe at the bottom of tank in order to prevent elvers swimming into the collectors. Mesh size was selected to be large enough to allow the passage of faeces into the faecal collectors. Two 32 mm PVC pipes prepared as parallel units were used to prevent elvers aggregating on the mesh. Before feeding, three 40 mm PVC pipes were plugged to prevent pellets going through the mesh. Flow rate into each tank was adjusted to 1.1 l/min and turned off during feeding. After feeding, the pipes were removed and all the uneaten food flushed out using the valves beneath the tanks. For each replicate, food consumption was measured on the second and fifth d of the faecal collection period. Approximately two thirds of the water volume in each tank was replaced with clean freshwater from a 1000-l reservoir tank. Elvers were prevented from escaping using mesh cloth under the lids. Over the experiment the water quality parameters were (mean ± SD): temperature, 26.1±0.3 C°; dissolved oxygen, 6.5±0.3 mg/l; pH, 6.9±0.4; total ammonia nitrogen, 0.12±0.03 mg/l. Photoperiod was 11h:13h Light:Dark.

6.2.2. Diet formulation and preparation

Eight test diets were formulated to contain 69% of a reference diet, 30% of the test ingredient and 1% chromic oxide as an inert marker (Cho et al., 1982)(Table 6.1). The ingredients tested for digestibility were: soybean meal, SBM (solvent extracted soybean, Pivot Aquaculture, Tasmania, Australia); canola meal, CM (solvent extracted, Pivot Aquaculture, Tasmania, Australia); corn gluten meal, CGM (Pivot Aquaculture, Tasmania, Australia); lupin meal, LM (whole Australian sweet lupin, *Lupinus angustifolius*, autoclaved at 105 °C for 10 min. and ground, Milne Feeds Pty.Ltd., Western Australia, Australia); field pea meal, FPM (whole field pea, *Pisum sativum*, autoclaved at 105 °C for 10 min. and ground, Milne Feeds Pty.Ltd., Western Australia, Australia); meat meal, MM (wet pressed and spray dried meat soluble, Daka, A.M.B.A Denmark); blood meal, BM (co-agulated, dried and ground, Peerless Holdings Pty.Ltd., Victoria, Australia) and poultry meal, PM (Edgell,

,Tasmania, Australia). The chemical composition of the test diets and ingredients is shown in Tables 6.2 and 6.3. The composition of the reference diet (REF3) was similar to one used by Carter, (1998) but contained dextrin instead of wheat flour as a carbohydrate source (Table 6.1). Fish meal and fish oil were from jack mackeral, *Trachurus picturatus* (Pivot Aquaculture, Tasmania, Australia). Vitamin and mineral mixtures included in the reference diet (REF3) were prepared according to De la Higuera et al. (1989) (Table 6.1).

Dry ingredients of the reference diet were mixed with a Hobart mixer for 30 min. fish oil and the vitamin and mineral mixtures were then added and the mixture was mixed for a further 20 min. The reference diet was stored at - 20°C until used. Whole ingredients and those containing large particles (≥ 0.5 mm) were ground (M20, IKA Labortechnik, Germany) before being mixed with the reference diet. After being combined with the reference diet, ingredients, chromic oxide (1%) and water (50 g/kg diet), each test diet was mixed for 30 min. Diets were manufactured as pellets (1 mm die) using a laboratory pellet mill (Model CL-2, California Pellet Mill Co., U.S.A). All the diets were dried overnight at 37 °C in a fan forced oven. Dried diets were individually bagged and stored at - 20 °C until used.

6.2.3. Sampling and calculation of apparent digestibility coefficients

Because the system was kept in a warm temperature controlled area, faecal samples were collected in 75 ml sample jars held in crushed ice filled foam boxes in order to prevent the bacterial degradation of the faeces. Faecal samples were collected from the settlement collector between 1800 and 0900 h on each day during each 7-d faecal collection period. During the collection, sample jars were carefully unscrewed from the collectors and frozen immediately at -20 °C without draining the excess water in jars. All the frozen sample jars were freeze dried. Following freeze drying, the faecal samples from each replicate tank of each treatment throughout the 7-d collection period were ground, pooled (by equal weight) and stored at - 20°C until analysis. Freeze dried samples were used in the analysis of marker, chromic oxide and nutrients (see below). The apparent digestibility coefficients for the reference diet and test diets were calculated using the standard formula;

$$ADC\ (\%) = 100-[100(\%I_{diet}/\%I_{faeces})\times(\%N_{faeces}/\%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N the nutrient. The ADC for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and energy (ADC_{kJ}) and for each ingredient was calculated as;

$$ADCi\ (\%) = ADC_{test}+((0.7\times N_{REF3})/(0.3\times Ni))\times(ADC_{test}-ADC_{REF3})$$

(Bureau et al., 1999) where ADCi is the apparent digestibility coefficients for each ingredient; ADC_{test} is the apparent digestibility (%) of the test diets; N_{REF3} is the nutrient content of the reference diet; Ni is the nutrient content of each test ingredient; ADC_{REF3} is the apparent digestibility of the reference diet.

6.2.4. Chemical analysis

Diets, ingredients and faeces were analysed for crude protein (Kjeldahl, selenium catalyst; %N×6.25) and gross energy (bomb calorimeter; Gallenkamp Autobomb, calibrated with benzoic acid). Crude fat in diets and ingredients was analysed according to the method of Bligh and Dyer (1959). Dry matter (g/kg DM) and ash in diets and ingredients were analysed using standard methods (AOAC, 1995). Chromic oxide was determined according to Furukawa and Tsukahara (1966).

6.2.5. Statistical analysis

Data are reported as ±S.E.M througout the text. The apparent digestibility coefficients for dry matter, crude protein and energy calculated for each of the test ingredient were arcsin-transformed prior to analysis and normality and homogeneity of variance were confirmed for each parameter (JMP Version 3.2.1). Means were compared by one-way ANOVA. When a significant treatment effect was observed a Tukey-Kramer HSD test was used to compare means. Significance was accepted at probabilities of 0.05 or less.

6.3. Results

All the test diets and the reference diet (REF3) were accepted well by the elvers and good feed consumption was found. There was no mortality throughout the experiment. Apparent digestibility coefficients for dry matter (ADC_{DM}) ranged between 37 and 93% among all the ingredients tested (Table 6.4). ADC_{DM} for plant proteins were more varied than those of animal by-products. FPM had a significantly lower ADC_{DM} than the other meals whereas CGM had a significantly higher ADC_{DM} than all the other meals except PM, MM and BM. There was no significant difference between ADC_{DM} values for SB and CM and these values did not significantly differ from the ADC_{DM} value for poultry meal. The ADC_{DM} of LM was the second lowest among the plant proteins and significantly different from both that of the plant proteins and animal by-products tested. The ADC_{DM} values for animal by-products (MM, PM and BM) ranged from 73.8 to 89.7% and they were higher than plant proteins except CGM and CM (Table 6.4). The highest ADC_{DM} was obtained on BM and it was significantly different than PM. However, there was no significant difference between the ADC_{DM} values of BM and MM. PM had the lowest ADC_{DM} between animal by-products and it was not significantly different than the ADC_{DM} of MM.

Apparent digestibility coefficients for crude protein (ADC_{CP}) of all the test ingredients varied between 85 and 97% (Table 6.4). It appeared that the variability of ADC_{CP} values for plant proteins and animal by-products was similar to each other (Table 6.4). The lowest ADC_{CP} was obtained on FPM and it was significantly lower than that of the other plant proteins tested except SBM. Although ADC_{CP} of FPM was significantly lower than that of the other plant proteins, the scale of the difference was not as large as ADC_{DM} or ADC_{kJ} among plant proteins (e.g 12% difference between the ADC_{CP} of FPM and CGM vs 56 % difference between ADC_{DM} in the same ingredients) (Table 6.4). CGM had the highest ADC_{CP} of all ingredients tested. However, there was no significant difference between ADC_{CP} of CGM and SBM, CM and LM. There was also no significant difference between ADC_{CP} of CGM and BM and MM. ADC_{CP} of CGM was, however, significantly higher than that of PM. ADC_{CP} of PM was the lowest between animal by-products tested. However, there was no significant difference between PM and MM. ADC_{CP}

of BM was the highest between animal by-products and significantly higher than that of PM. ADC_{CP} of BM did not significantly differ from ADC_{CP} of plant proteins except FPM (Table 6.4).

Apparent digestibility coefficients for energy (ADC_{kJ}) followed a similar trend to ADC_{DM} both in plant proteins and animal by-products. ADC_{kJ} of plant proteins were significantly lower than that of animal by-products except PM (Table 6.4). The variability of ADC_{kJ} between plant proteins was far greater than the variability of ADC_{kJ} between animal by-products (Table 6.4). The highest ADC_{kJ} was obtained on CGM and it was significantly higher than that of the rest of plant proteins tested. FPM had the lowest ADC_{kJ} digestibility of all the ingredients tested and it was significantly lower than that of both the other plant proteins and animal by-products. LM had the second lowest ADC_{kJ} among all the ingredients tested and was significantly lower than that of CGM, SBM, CM and BM, MM and PM. SBM and CM had similar ADC_{kJ} to each other eventhough they were significantly lower than CGM and all the other animal by-product ingredients (MM, PM and BM). Although the ADC_{kJ} of BM was significantly higher than that of MM and PM, the difference between the ADC_{kJ} of BM and PM was almost 2 fold higher than the difference between the ADC_{kJ} of BM and MM. There was no significant difference between the ADC_{kJ} of MM and PM (Table 6.4).

6.4. Discussion

6.4.1. Methodology

The body shape of anguillids makes the use of settlement faecal collectors without a modification difficult in digestibility studies with eels. Besides body shape, the size of the fish used in digestibility studies determines the selection of faecal collection methods. Stripping and the dissection of the anterior or posterior sections of the intestinal tract were demonstrated to be an ineffective feacal collection method with smaller size of fish (Cho et al., 1982; Allan et al., 1998). Settlement allows digestibility to be measured with smaller fish and causes minimal disturbance to faeces during collection.

Table 6.1

Formulation (g/kg) and chemical composition of the reference diet (REF3)

Ingredients	REF3
Fish meal	600.0
Fish oil	170.0
Dextrin	210.0
CMC	10.0
Minerals[1]	5.0
Vitamins[2]	5.0
Chemical composition (g/kg DM)	
Moisture	116.7±1.2
Crude protein	411.9±1.8
Crude fat	256.8±7.9
Ash	96.5±1.6
GE (MJ/kg)	21.0±0.04

[1]Mineral mixture (g/kg food): According to De la Higuera et al. (1989): CaH_2PO_4;1.37, $CaCO_3$; 1.306, KH_2PO_4; 0.954, KCl; 0.096, NaCl; 0.577, $MnSO_4.H_2O$; 0.036, $FeSO_4.7H_2O$; 0.144, $MgSO_4$; 0.48, KI; 0.0018, $CuSO_4.5H_2O$; 0.0048, $ZnSO_4.7H_2O$; 0.024, $CoSO_4$; 0.0028, Na_2MoO_4; 0.0008, Na_2SeO_3; 0.002, $AlSO_4.18H_20$; 0.0016.

[2]Vitamin mixture (g/kg food): According to De la Higuera et al. (1989): Calcium pantothenate; 0.13, Thiamine; 0.044, Riboflavin; 0.109, Pyridoxine; 0.033, Inositol; 0.874, Biotin; 0.001, Folic acid; 0.011, Choline chloride; 2.623, Nicotinic acid; 0.219, Cyanocobalamin; 0.002, Ascorbic acid; 0.874, Retinol; 0.044, Menadione; 0.022, ∝-tocopherol; 0.007, Cholecalciferol; 0.009. Individual ingredients were supplied by Sigma-Aldrich Pty.Ltd. and ICN Biochemicals Pty.Ltd. Australia.

Table 6.2

Chemical composition (g/kg DM) and energy content of test diets used in the digestibility experiment

	Diets							
	SBM	CM	CGM	LM	FPM	MM	BM	PM
Dry matter	873.9±1.2	878.2±2.1	881.6±2.7	882.6±2.6	872.1±2.1	866.2±2.5	887.1±3.9	879.1±1.3
Crude protein	434.0±0.0	399.0±2.8	471.0±2.8	396.5±4.9	352.5±19.1	484.5±9.2	562.5±0.7	450.5±28.9
Crude fat	199.0±0.3	191.4±9.9	215.6±13.0	210.8±0.6	197.6±0.6	210.2±0.3	185.8±5.9	318.2±16.7
Ash	88.3±1.7	86.6±0.2	78.9±2.1	77.4±1.7	76.2±0.6	91.3±1.6	75.2±0.8	91.9±0.2
NFE[a]	278.7±0.9	323.0±4.9	234.5±5.1	315.3±2.2	373.7±10.6	214.0±4.8	176.5±3.0	139.4±14.4
GE (MJ/kg DM)	20.4±0.1	20.0±0.0	21.1±0.2	20.3±0.007	19.7±0.05	20.6±0.04	21.4±0.06	22.2±0.007

[a]Calculated as the remainder of crude protein+crude fat+ash (Carter and Hauler, 2000).

Table 6.3

Chemical composition (g/kg), gross energy (MJ/kg) and the essential amino acid content (g/kg) of the ingredients used in the digestibility experiment

	Ingredient							
	SBM	CM	CGM	LM	FPM	MM	BM	PM
Dry matter	880.3±9.1	918.1±10.8	915.8±5.7	921.4±2.9	891.8±9.1	825.5±6.6	962.4±7.8	916.9±6.2
Crude protein	515.9±3.1	423.8±4.5	679.7±7.5	298.5±5.7	247.6±19.4	744.7±5.8	958.2±13.2	452.5±5.2
Crude fat	40.3±0.4	49.6±0.7	86.0±1.1	78.3±0.2	26.1±0.7	140.3±0.5	28.8±0.4	454.9±11.9
Ash	89.1±17.4	108.8±9.5	96.9±31.1	119.8±0.8	63.9±6.6	154.4±14.2	53.2±2.7	99.2±26.4
GE (MJ/kg)	18.6±0.3	17.5±0.3	21.9±0.7	18.2±0.4	17.2±0.1	20.6±0.1	23.2±0.03	26.6±0.1
Essential Amino Acids (EAA)[1]								
Arginine	37.6±0.9	24.7±0.2	22.8±0.2	43.4±3.8	11.8	54.2±1.3	43.9±0.6	41.3±0.07
Histidine	12.5±0.8	8.9±0.1	13.4±0.1	9.0±0.9	26.2	11.2±1.5	55.0±0.4	9.3±0.5
Isoleucine	23.5±0.6	16.1±0.1	28.5±0.1	14.9±1.3	6.3	18.5±1.3	15.1±0.2	22.6±0.1
Leucine	38.3±1.0	27.7±0.1	112.4±0.6	23.4±1.8	11.2	39.3±1.7	113.3±0.6	40.3±0.3
Lysine	30.5±0.1	18.8±0.07	9.2±0.7	15.4±1.4	18.8	30.9±1.4	81.0±2.8	28.5±1.2
Methionine	5.1±0.3	7.6±0.07	16.3±0.1	2.3±0.2	17.0[a]	8.4±0.1	14.6±0.07	9.0±0.6
Phenylalanine	25.2±0.6	15.9±0.1	43.3±0.1	14.2±1.0	3.6[b]	19.1±1.2	64.9±0.1	23.5±0.3
Threonine	21.0±0.6	18.1±0.2	24.1±0.1	12.4±1.0	NA	21.6±1.2	49.2±0.3	23.7±0.3
Tryptophan	4.8	2.7	1.5	2.6	NA	4.5	6.0	4.3
Valine	24.4±0.6	20.6±0.1	30.9±0.1	14.0±1.0	21.8	26.8±0.5	79.0±0.1	29.9±0.1

[a]includes cysteine

[b]includes tyrosine

[1]Carter, unpublished

Table 6.4

Apparent digestibility coefficients (%) for dry matter (DM), crude protein (N) and energy (kJ) for the Australian short-finned eel fed a variety of plant and animal proteins

Parameter	Ingredient								*P*
	SBM	CM	CGM	LM	FPM	MM	BM	PM	
ADC_{DM}	69.8 ± 1.7^{c}	76.8 ± 0.9^{cd}	92.8 ± 1.0^{f}	57.0 ± 2.6^{b}	37.1 ± 6.9^{a}	82.4 ± 3.9^{de}	89.7 ± 0.6^{ef}	73.8 ± 5.6^{cd}	< 0.0001
ADC_{N}	90.5 ± 1.6^{ab}	94.2 ± 0.2^{b}	97.0 ± 0.5^{b}	96.2 ± 3.2^{b}	84.9 ± 3.4^{a}	92.1 ± 0.4^{ab}	96.1 ± 2.5^{b}	86.9 ± 3.8^{a}	< 0.001
ADC_{kJ}	75.6 ± 3.1^{c}	77.3 ± 1.0^{c}	96.8 ± 1.1^{e}	60.8 ± 1.4^{b}	46.4 ± 6.8^{a}	88.5 ± 4.1^{d}	96.6 ± 0.8^{e}	82.1 ± 5.6^{cd}	< 0.0001

Each value is the mean (± S.E.M) of three replicates

Means with different letter in the same row are significantly different (Tukey-Kramer HSD multiple comparison)

Table 6.5

Digestible nutrient contents (g/kg DM) for ingredients consumed by the Australian short-finned eel.

	Ingredient							
	SBM	CM	CGM	LM	FPM	MM	BM	PM
Dry matter	614.4±6.4	705.1±8.3	849.9±5.2	525.2±1.6	330.8±3.4	680.3±5.6	863.3±7.0	676.6±4.5
Crude protein	467.0±2.8	399.2±4.2	659.4±7.3	287.1±5.5	210.2±16.5	685.9±5.3	920.8±12.7	422.0±4.7
GE (MJ/kg DM)	14.1±0.2	13.5±0.3	21.2±0.7	11.1±0.2	8.0±0.1	18.3±0.1	22.4±0.1	21.9±0.1
Essential Amino Acids (EAA)[1]								
Arginine	34.0±0.8	23.2±0.2	22.1±0.2	41.8±3.6	10.0	49.9±1.2	42.2±0.6	35.9±0.1
Histidine	11.3±0.7	8.4±0.1	13.0±0.1	8.6±0.8	22.2	10.3±1.3	52.9±0.4	8.0±0.4
Isoleucine	21.2±0.6	15.2±0.1	27.6±0.1	14.4±1.2	5.3	17.1±1.2	14.5±0.2	19.7±0.1
Leucine	34.7±1.0	26.1±0.1	109.0±0.6	22.6±1.8	9.5	36.2±1.6	108.9±0.6	35.0±0.3
Lysine	27.6±0.1	17.7±0.1	9.0±0.6	14.9±1.3	16.0	28.5±1.3	77.8±2.7	24.8±1.1
Methionine	4.6±0.3	7.2±0.1	15.8±0.1	2.2±0.2	14.4	7.7±0.1	14.0±0.1	7.9±0.5
Phenylalanine	22.8±0.6	15.0±0.1	42.0±0.1	13.6±1.0	3.1	17.6±1.1	62.4±0.1	20.4±0.3
Threonine	19.0±0.6	17.0±0.1	23.4±0.1	12.0±0.9	NA	19.9±1.1	47.3±0.3	20.6±0.3
Tryptophan	4.3	2.5	1.46	2.5	NA	4.1	5.8	3.7
Valine	22.0±0.6	19.4±0.1	29.9±0.1	13.5±0.9	18.5	24.7±0.5	75.9±0.1	26.0±0.1

[1] Values calculated assuming approximation of the availability of amino acids to crude protein digestibility coefficients in each ingredient (Yamamoto et al., 1997)

Table 6.6

Apparent digestibility coefficients calculated for same type of plant and animal proteins by several Australian warmwater species (Silver perch, *Bidyanus bidyanus* and the short-finned eel, *A. australis australis*.

	Ingredients				
Parameter	SBM	CGM	BM	MM	Reference
Silver perch					
ADC_{DM}	80.5±1.7	98.3±2.0	98.7±1.7	NA	Allan et al. (1999)[1]
ADC_{N}	95.4±0.7	97.7±0.2	92.4±2.6	NA	"
ADC_{kJ}	83.3±1.6	96.4±0.3	104.3±8.1	NA	"
short-finned eel					
ADC_{DM}	81.6±2.25	NA	NA	33.4±3.49	De Silva et al. (2000)[2]
	69.8±1.7	92.8±1.0	89.7±0.6	82.4±3.9	The present study
ADC_{N}	91.6±1.28	NA	NA	53.0±3.90	De Silva et al. (2000)[2]
	90.5±1.6	97.0±0.5	96.1±2.5	92.1±2.5	The present study
ADC_{kJ}	56.2±5.9	NA	NA	63.8±5.36	De Silva et al. (2000)[2]
	75.6±3.1	96.8±1.1	96.6±0.8	88.5±4.1	The present study

[1] Faeces from juvenile silver perch (9.8-11.2 g) were collected by settlement over 18 h on each day of 12 d faecal collection period

[2] Faeces from medium size shortfinned eel (average 40 g) were collected by siphoning between 1830 and 0830 h on each day of faecal collection period

To minimise the breakage and leaching of nutrients from faeces that may result in a significant amount of overestimation of digestibility coefficients, modified Guelph-type of settlement collectors held in crushed ice were used in the present study. Because similar digestibility coefficients for eels (Schmitz et al., 1984) and other fish species like the Australian silver perch (Allan et al., 1998) and trout (Yamamoto et al., 1998) were reported for the same type of ingredient using almost similar or exactly the same type of collection technique to the present study, the validity of the technique could be justified by being comparable to many other studies.

One of the biggest difficulties involved with fish digestibility studies is to determine the correct time for faeces collection. In order to prevent leaching of nutrients, faeces must be collected over the shortest possible time after being voided by the fish in settlement type collectors. In the present experiment the sample size for the chemical analysis was large enough before the morning's feed and the collection time was set at 12 h after the evening meal (1700-1800 h). Previous studies have also used the same time frame for the collection of faeces in digestibility studies with eels (De Silva et al., 2000; Tibbetts et al., 2000). In contrast to findings by Watanabe et al.(1996), Allan et al. (1998) demonstrated that prolonged collection (18 h following previous meal) of faeces in collectors when held in ice caused negligible leaching of dry matter and protein.

Since very few potentially useful ingredients can be fed as the sole component of a diet, it is almost necessary to blend these ingredients with a reference diet to determine their digestibility values for a particular finfish species (usually 70:30 % of the reference diet and test ingredients) (Cho et al., 1982; da Silva and Oliva-Teles, 1998). However, this ratio may be 85:15 % of a test diet if fish have difficulty accepting the diet (De Silva and Anderson, 1995; da Silva and Oliva-Teles, 1998). Although this procedure assumes that there are no significant interactions among ingredients in their digestibility, this assumption may not always be true especially when feed ingredients are rich in carbohydrates or when antinutritional factors are present (Kaushik and Médale, 1994; Gomes da Silva and Oliva-Teles, 1998). There are very few studies which specifically targeted this issue in fish digestibility studies. However, da Silva and Oliva-Teles (1998) found that there were no significant differences in the ADC of protein and energy of two fish meals (standard fish meal

from Norway and a Portugese brown fish meal), a fish protein hydrolysate, blood meal, meat meal soybean meal and yellow dextrin at two inclusion levels (15 and 30 %) investigated. Studies with salmon and trout have also cited the indigestibility of the added filler materials or increased susceptibility to Maillard type reaction between the free amino acids in fish silage and the carbohydrate in the dry additives for lower weight gains with co-dried fish silage diets (Hardy et al., 1983;1984). In this way, the reduced nutritional quality of diets could occur due to the presence of relatively high levels of free amino acids whose absorption may not be synchronised with protein synthesis or energy absorption (Fagbenro et al., 1994).

6.4.2. Animal proteins

The Australian short-finned eel digested the dry matter and energy in animal by-products significantly better than most of the plant proteins. Although not significantly different, the apparent digestibility coefficients of meat meal (MM) for dry matter, crude protein and energy were higher than that of poultry meal (PM) but lower than that of blood meal (BM). The only animal-by product tested for digestibility in the short-finned eel has been the meat meal (De Silva et al., 2000). Their result was in contrast to what was reported in the present study (82 and 89% dry matter and energy digestibility coefficients of meat meal, respectively) and relatively high ash content (29.4% DM) of meat meal may have caused lower dry matter and energy digestibilities in their study. Similar observations were made with salmonids and concluded that a large amount of poorly digested ash in the meat or meat and bone meal results in markedly lower dry matter digestibility (Cho et al., 1982; Bureau et al., 1999). Protein digestibility of meat meals appears not to be related to the amount of ash in the products but a slight increase in protein digestibility of meat meal products was observed with air classification process as a reduction in collogen content in these products (Bureau et al., 1999).

Although there is no available eel digestibility results for poultry and blood meal in the literature to compare with, our findings are in line with what was reported with salmonid digestibility values for these product (Hajen et al., 1993; Pfeffer et al., 1995; Suguira et al., 1998; Bureau et al., 1999). It appears that a significant improvement in the crude protein digestibility has occurred over the years through

better manufacturing practises (Miller, 1996). Spray-dried blood meal gave high digestibility values in this study and this was in line with findings with other studies (Cho et al., 1982). Compared to spray-drying, other processing techniques available to produce blood meal like rotoplate, steam-tube and ring-drying were shown to give a significantly lower digestibility coefficients in fish due to excessive heat damage to proteins (Cho et al., 1982).

6.4.3. Plant proteins

Apparent protein digestibility coefficients for plant proteins ranged between 85 and 97% in the present study. This was in agreement with findings from previous studies which consistently reported high levels of digestive utilisation of plant proteins by carnivorous and omnivorous fish, although plant proteins often have lower protein contents than animal by-products(Cho and Cowey, 1991; McGoogan and Reigh, 1996). However, plant proteins contains high levels of complex carbohydrates and several anti nutritional factors like trypsin inhibitor which may be detrimental for fish growth (Wilson and Poe, 1985). It is well documented that the ability to utilise plant carbohydrates as energy sources varies among species and it is rather limited in many carnivorous fish (Cho et al., 1982; Cowey and Walton, 1989; Kaushik et al., 1989; Morales et al., 1994; Wilson, 1994; García-Gallego et al., 1995). The significantly lower energy and dry matter digestibility of lupin and pea meals than that of other plant proteins, found in the present study, seem to be associated with the quantity and the chemical composition of the carbohydrates they contain (McGoogan and Reigh, 1996). Previously, soybean meals have been shown to have equal ADC_{CP} as fish meal but had lower ADC_{DM} in eels (Schmitz et al., 1984). Adult European eels (weighing between 170-230 g) had ADC_{DM} of 68% from soybean meal compared with the 87% from fish meal (Schmitz et al., 1984). In contrast to 70 and 76% ADC_{DM} and ADC_{kJ} for a soybean meal reported in this study, De Silva et al. (2000) demonstrated that ADC_{DM} and ADC_{kJ} of soybean meal were 82 and 56%, respectively in the Australian short-finned eel weighing about 40 g. It is likely that faeces collected with disturbance (siphoning) and held in tanks for a period without cooling had an impact and resulted in higher ADC_{DM} and ADC_{kJ} of soybean meal in their study. Cooling the faeces after settlement in collectors was shown to prevent bacterial decomposition of faeces hence decreasing the chance of

having overestimated digestibility coefficients (Spyridakis et al., 1989). However, almost 20 % lower ADC_{kJ} of soybean meal reported by De Silva et al. (2000) must also be related to the quality of the protein source. Therefore, digestibility coefficients reported for the same type of meals in different studies are currently hard to compare since values are affected by the techniques used to measure digestibility, the quality of the ingredients, dietary composition, fish size, ration level and the water temperature employed in the experiment (Wilson and Poe, 1985; Anderson et al., 1992; Watanabe et al., 1996; Yamamoto et al., 1997; da Silva and Oliva-Teles, 1998; Bureau et al., 1999).

Apparent digestibility coefficients for dry matter, crude protein and energy in corn gluten were over 90 % in the present study. Corn gluten is a major co-product of corn wet milling and contains high protein and low fiber (Park et al. 1997). Although there is no published apparent crude protein digestibility values of corn gluten for eels, generally high values were reported with other carnivorous species like salmonids (Cho and Slinger, 1979; Morales et al. 1994; Yamamoto et al. 1997,1998a; Suguira et al. 1998) and red sea bream (Yamamoto et al. 1998a). Yamamoto et al. (1997) reported that a protein digestibility was 96% for corn gluten meal in fingerling rainbow trout at 15 °C and demonstrated that the availabilities of amino acids from corn gluten meal almost approximated to the apparent protein digestibility value. A similar assumption was made in order to calculate the digestible essential amino acid content of each protein source in Table 6.5. Although there are not many apparent dry matter and energy digestibilities reported for corn gluten in different species, 93 and 97% dry matter and energy digestibility values found in the present study were comparable to the results obtained with the Australian silver perch (Allan et al. 1998). However lower corn gluten lipid and carbohydrate digestibilities were shown in rainbow trout (Morales et al. 1994). This may not be surprising since the European eel has been shown to have a comparatively greater ability to utilise high levels (over 30% of diets) of corn starch in balanced diets than the rainbow trout (García-Gallego et al. 1995). Warm water fish species are able to utilise much higher levels of dietary carbohydrate than cold water or marine fish due possibly to higher amylase activity present in the digestive system of these fishes (Wilson, 1994). However, diets with high levels of crude or raw starch was shown to inhibit the digestibility of these diets by the European eel

(Spannhof and Kühne, 1977) not because of a decrease in amylase secretion rate but an increase chance of adsorbtion of the amylase to the crude or raw starch, thus inhibiting starch hydrolysis (Spannhof and Plantikow, 1983). Better digestive utilisation of dietary ingredients would promote greater efficiency in the utilisation of dietary protein and energy and result in lower waste production (Kaushik and Médale, 1994; Morales et al. 1994; Robaina et al., 1997). Therefore, more research is needed in order to understand the dietary energy efficiency of the practical diets containing corn gluten meal for the Australian short-finned eel since the dietary gross energy efficiency to promote energy retention and growth is directly related to the energy utilisation at the digestive level.

6.4.4. Summary and conclusion

The present study demonstrated that the juvenile Australian short-finned eel digested the dry matter and energy in animal by-products better than in plant proteins with the exception of corn gluten. However, apparent crude protein digestibility coefficients of the ingredients tested ranged between 85 and 97% and there were smaller differences between plant proteins and animal by-products. Both high levels of complex carbohydrates (olligosaccharides) and anti nutritional factors in raw and autoclaved lupin and field pea meals might have caused significantly lower dry matter and energy digestibilities in juvenile short-finned eel. These results were in agreement with those reported for other carnivorous fish species such as salmonids (Watanabe et al., 1996; Yamamoto et al., 1997; Storebakken et al., 1998; Suguira et al., 1998), hybrid striped bass (Sullivan and Reigh, 1995), red drum (McGoogan and Reigh, 1996) or an eel species (Schmitz et al., 1984).

The digestibility coefficients for separate ingredients are assumed to be additive and can be used in the least cost diet formulations for fish species (Allan et al., 1998). The effectiveness of diets formulated upon the basis of digestibilities of the nutrients and energy in individual ingredient can be evaluated by observation of weight gain, feed efficiency and body composition of fish receiving the diets under particular culture regimes (Cho et al., 1982). It is a necessity to conduct growth trials with potential alternative protein ingredients (decided upon their digestibility values or the availability of amino acids to a particular fish specie) for the success of feed

development studies. This research successfully identified the highly digestible Australian plant and animal proteins that may replace the fish meal protein in balanced diets for juvenile Australian eel and more research is needed to achieve it.

CHAPTER SEVEN

Fish meal replacement by plant and animal by-products in diets for the Australian short-finned eel, *Anguilla australis australis* (Richardson)

7.1. Introduction

Fish meal is a widely used and expensive protein component of fish diets (Naylor, 2000; Carter and Hauler, 2000; De Silva et al., 2000). Since the production of fish meal almost completely relies on wild catch, variability in catch rates makes the price and availability of fish meal unreliable (Davies and Morris, 1997). Therefore, the partial replacement of fish meal in fish diets with cheaper and more reliable alternative protein sources without compromising growth has been a priority in aquaculture nutrition research (Morales et al., 1994; Hardy, 1996).

Besides price and availability, the quality of the protein (availability and balance of amino acids) is an important determinant of the efficiency of deposition of dietary nitrogen within the body (Boorman, 1980; Kaushik, 1990; Refstie, 1997; García-Gallego et al., 1998). Soybean has been the major alternative protein source tested for fish meal replacement studies with fish species, particularly salmonids, since it has a high protein content and a good balance of essential amino acids (Pongmaneerat and Watanabe, 1993; Carter et al., 1994; Olli et al., 1994; Davis et al., 1995; Olli and Krogdahl, 1995; Robaina et al., 1995; Nengas et al., 1996; Refstie et al., 1998; Carter and Hauler, 2000). The research on fish meal protein replacement with other potential plant and animal proteins has been limited due to their unbalanced amino acid profiles and low level of proteins (Carter and Hauler, 2000). Apart from amino acid balance, many of the plant proteins contain antinutritional factors like protease inhibitors and complex carbohydrates (olligosaccharides) which can impair the growth performance and nutrient utilisation in monogastric animals including fish (Wilson and Poe, 1985; Dabrowski et al., 1989; Hernandez-Infante et al., 1998). However, recent developments in mechanical processing technology have minimised the influence of major antinutritional factors (particularly enzyme inhibitors) resulting in significant improvements in the digestibility of protein and energy in plant proteins (Rumsey et al., 1993; Hernandez-Infante et al., 1998).

Compared to salmonids less attention has been paid to the partial replacement of fish meal in diets for other important farming species like eels. Soybean (Degani, 1987), fish silage (Gonçalves et al., 1989), blood meal (Lee and Bai, 1997) and meat meal and sunflower meal (García-Gallego et al., 1998) have all been tested for the

partial replacement of fish meal in the diets of different eel species. Results suggest that higher substitution of fish meal in eel diets might be possible by supplementation of EAA (Essential Amino Acids) to restore the amino acid profile of the feed to a level which matches the requirement of the target species (Davies and Morris, 1997).

Australia has a relatively wide range of plant and animal-by products to be utilised in fish diets. Therefore, this study aimed to demonstrate the effects of substituting 23% of fish meal protein with protein from Australian soybean meal, lupin meal, corn gluten meal and meat meal in nutritionally balanced experimental diets on growth and growth efficiency of juvenile Australian short-finned eels. Blood meal, DL-methionine and L-tryptophan was used to bring the essential amino acid profile of each experimental diet in line with the requirement of the Japanese eel (Arai, 1991).

7.2. Materials and methods

7.2.1. Fish and maintenance

Australian short-finned eel elvers, randomly selected from holding tanks, were acclimatised to the experimental system for a week. During acclimatisation elvers were fed the commercial eel diet twice a day. After this period all the elvers were taken out, pooled and randomly re-allocated to each carboy. Each treatment (diets containing different alternative protein sources) was replicated three times and twenty fish (average wet weight of 2.23±0.4 g) per carboy were used in the experiment.

Uneaten food and faeces were removed from carboys daily by siphoning (see Chapter 2.4). Mean (±SD) values of water quality parameters were recorded throughout the experiment; 25.8±0.6 °C water temperature (daily), 6.9±0.3 mg/l DO (twice a week), 6.96±0.4 pH (twice a week), 0.21±0.06 mg/l ammonia-nitrogen (NH_3-N) (twice a week) and 0.011±0.007 mg/l nitrite-nitrogen (NO_2-N) (twice a week). Photoperiod was 11h:13h Light:Dark.

7.2.2. Diet formulation and preparation

Four experimental diets were formulated to replace 23% of total crude protein with one of several plant or animal ingredients: soybean meal, SBM (solvent extracted soybean, Pivot Aquaculture, Tasmania, Australia); lupin meal, LM (ground whole Australian sweet lupin, *Lupinus angustifolius*, Milne Feeds Pty.Ltd., Western Australia, Australia); meat meal, MM (wet pressed and spray dried meat solubles, Daka, A.M.B.A Denmark); and corn gluten meal, CGM (Pivot Aquaculture, Tasmania, Australia). Fish meal and fish oil in all the diets were from jack mackeral, *Trachurus picturatus* (Pivot Aquaculture, Tasmania, Australia). Dextrin was used as a carbohydrate source. Vitamin and mineral mixtures included in the diets were prepared according to De la Higuera et al. (1989) (Table 7.1).

The crude protein content from fish meal and blood meal contributed equally to total crude protein content in each diet. Diets were, principally, formulated to be isonitrogenous and isoenergetic. However, consideration was given to make the total digestible amino acid contents similar meeting the estimated essential amino acid requirement of the Japanese eel as a reference (Arai, 1991). In this regard, all diets were supplemented with blood meal (co-agulated, dried and ground, Peerless Holdings Pty.Ltd., Victoria, Australia) to ensure phenylalanine was in excess of requirements. For the same reason DL-methionine and L-tryptophan (Sigma-Aldrich Pty.Ltd. Australia) were also added to the diets. The control diet (CON) was fish meal based and contained same amount of blood meal as the other diets (Table 7.1). However, diets LM and CGM contained slightly lower amount of blood meal than 10 % in other diets due to protein content adjustments.

Before being mixed with the rest of the dry ingredients, whole kernels of lupin were ground to ≤ 0.1 mm (M20, IKA Labortechnik, Germany). Pellets were made as described previously (Chapter 6.2.2). Dry ingredients of the diets were mixed with a Hobart mixer for 30 min., fish oil and the vitamin and mineral mixtures were then added and mixed for a further 20 min. Water (50 g/kg) was added to diets before pelleting (1 mm die) using a laboratory pellet mill (model CL-2, California Pellet Mill Co., U.S.A). All diets were dried overnight at 37 ° C in a fan forced oven. Dried diets were individually bagged and stored at - 20 ° C until used.

7.2.3. Experimental procedure

Elvers were fed twice a day from 0900 to 1000 h and 1700 to 1800 h on rations equal to 5 % BW/d for 63 d. After the morning feed, the remaining half of the ration was stored at -20 ° C until the afternoon feed. Elvers were batch weighed every 3 weeks throughout the experiment and the ration adjusted accordingly. Elvers were anaesthetised (80 mg/l, Benzocaine) during weight and length measurements and vigorous aeration was used during recovery from the anaesthesia. The weight and length of individual elvers were measured at the beginning and end of the experiment.

Food consumption was measured once per week. The night before, all tanks were cleaned of solid organic material and food consumption (g DM) was measured the following day (see Chapter 5.2.3).

At the beginning and end of the experiment, samples of elvers were killed with benzocaine for whole body chemical analysis (see below). An initial group of 15 elvers was randomly selected from stock tanks, killed and stored at -20 ° C until analysis. Three elvers from each carboy were also sampled at the end of the experiment for the final whole body chemical composition.

7.2.4. Nitrogenous excretion

Daily ammonia- and urea-nitrogen excretion rates in all treatments were measured during the third week of the experiment. Sampling of carboys for ammonia- and urea-nitrogen was according to Engin and Carter (2001). The concentration of ammonia in samples was determined by the phenol-hypochloride method (Solorzano, 1969). Urea was analysed by the urease method (Elliott, 1976). Total ammonia-nitrogen concentration was calculated using a standard curve prepared from ammonium chloride solution. The difference between ammonia concentration before and after urease treatment was used to calculate urea concentration.

7.2.5. Apparent digestibility

Apparent digestibility coefficients (ADC) were measured at the end of the experiment. Because the number of elvers in each replicate tank was not sufficient to collect enough faecal matter for the chemical analysis, fish were pooled from the replicate tanks in each treatment. These treatment groups were then housed in the three tank modified Guelph settlement faecal collector (see Chapter 2.1). Elvers were fed the experimental diets containing chromic oxide (10 g/kg) for 10 d. On last five days of the feeding period, faecal samples from tanks were collected from the settlement collector between 1800 and 0900 h, freeze dried, ground, pooled (by equal weight) and used in the analysis of the marker, chromic oxide and nutrients. The ADC were calculated using the standard formula

$$ADC\ (\%) = 100 - [100(\%I_{diet}/\%I_{faeces}) \times (\%N_{faeces}/\%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N the nutrient.

7.2.6. Chemical analysis

Diets, faeces and dry whole body homogenates were analysed for crude protein (Kjeldahl, selenium catalyst; %N×6.25). Gross energy in diets and faeces was analysed by a bomb calorimeter (Gallenkamp Autobomb, calibrated with benzoic acid). Crude fat in diets and dry whole body homogenates were analysed according to the method of Bligh and Dyer (1959). Dry matter (g/kg DM) and ash in diets were analysed using standard methods (AOAC, 1995). Chromic oxide was determined according to Furukawa and Tsukahara (1966).

7.2.7. Elver growth performance evaluation

Following parameters were used to evaluate elver growth performance; Weight gain as WG = (final total tank weight-initial total tank weight); Specific growth rate as SGR (%/d) = [(ln final weight-ln initial weight)×100]/d; Feed efficiency ratio as FER = total weight gain (g)/total feed consumption (g DM); Protein efficiency ratio as PER (%) = [gain in weight (g)/protein intake (g)]×100; Productive protein value

as PPV (%) = [protein retained (g)/protein intake (g)]×100.

7.2.8. Statistical analysis

All data were subjected to one-way ANOVA using JMP 3.0 (SAS Institute) and reported as mean ± S.E.M throughout the text. Before proceeding with ANOVA, assumptions of normality and homogeneity of variance were confirmed using Shapiro-Wilk (Zar, 1996) and Cochran's (Underwood, 1981) tests, respectively. When a significant treatment effect was observed a Tukey-Kramer HSD test was used to compare means. Significance was accepted at the probability of 0.05 or less.

7.3. Results

7.3.1. Growth performance

There was no mortality in the experiment. However, escapees were treated as mortalities throughout the experiment (Table 7.2). The total final weight and total weight gain (g) were significantly higher for elvers fed the MM diet than for those fed LM diet. However, there was no significant differences between the diets MM and CON, SBM, CGM. Although the total weight gain obtained on LM was the lowest among the experimental diets there was no significant difference between LM and CON, SBM and CGM (Table 7.2). The trend in final total weight gain was reflected in SGR (%/d) values of treatments, the value for MM being significantly higher than that for LM. Although LM had the lowest SGR value, it was not statistically different from that of CON, SBM and CGM.

Weight increased in each treatment during the experiment (Figure 7.1). One replicate tank in each MM and CGM treatments showed faster growth than the other two replicate tanks. However this trend was less apparent during the last three-week time period of the experiment for MM than for CGM diet. Feed consumption, calculated as total feed, was not different between treatments with only elvers fed MM having, although not significantly, higher feed consumption (Table 7.2). However, the differences in both feed consumption and growth resulted in significant differences in feed efficiency ratio (FER), protein efficiency ratio (PER)

and productive protein value (PPV) (Table 7.2). The highest FER was obtained on MM and it was significantly higher than that of LM (Table 7.2). The FER for CON, SBM and CGM was almost similar and were not significantly different from either the MM or LM diet. Protein efficiency ratio (PER) and protein productive value (PPV) values exhibited a similar trend among treatments (Table 7.2). MM diet resulted in the highest PER and PPV values. The PER and PPV values were almost similar for the diets CON, SBM and CGM and did not differ significantly from that of MM diet. The lowest PER and PPV values were obtained on LM diet and were significantly lower than for CON, SBM, and MM (Table 7.2).

There were no statistically significant differences in chemical composition of fish among treatments and mean values were 55-57% for crude protein, 26-28% for crude lipid and 28-29% for dry matter (Table 7.3).

7.3.2. Apparent digestibility coefficients

Experimental diets significantly influenced apparent digestibility coefficients of crude protein (ADC_N) and energy (ADC_{kJ}) in the present study (Table 7.4). However, apparent digestibility coefficients of dry matter were not significantly different among diets (Table 7.4). The highest two ADC_N were obtained on SBM and LM and they were significantly different from CON, CGM and MM (Table 7.4). There were no significant differences between ADC_N values of CON, SBM and MM. ADC_{kJ} values of experimental diets varied between 76 to 85% in the present experiment. The lowest ADC_{kJ} was obtained on LM diet and it was significantly different from all the other treatments (Table 7.4).

7.3.3. Nitrogenous excretion

Ammonia-nitrogen (NH_3-N) excretion began to increase 4 h after the morning feed in all treatments (Figure 7.2). However, the excretion rate in MM diet at this hour was higher than plant protein containing and control diets (CON). Overall excretion rates were also higher in CON and MM diets. Although elvers were fed twice a day, only one peak 4 h following morning peak was evident in the daily ammonia-nitrogen excretion pattern. The lowest daily excretion rate was obtained on

LM diet.

Daily urea excretion was significantly affected by the experimental diets. 4 h following the afternoon feed, rates were 2-3 times higher in all the diets except CGM (Figure 7.3). Rates quickly returned to pre-feeding levels in all the treatments except in CON diet which retained a high level of excretion for a further 4 h (Figure 7.2).

7.4. Discussion

The present study demonstrated that fish meal protein could be replaced by corn gluten meal (CGM), soybean meal (SBM) and meat meal (MM) without comprimising the growth rates in the Australian short-finned eel. The level of substitution was 23% of fish meal protein in all the experimental treatments and it appeared that this level of substitution with whole lupin seed meal (LM) depressed growth and growth efficiencies in the short-finned eel. There is considerable benefit related to the replacement of part of the fish meal in commercial eel feeds, since more than 100 000 tons of eels are produced anually around the world (Zeller and Beumer, 1996).

Despite a considerable amount of research on the effects of fish meal protein replacement with soybeans (both processed and protein concentrate meals) on growth and growth efficiencies in other important aquaculture species like salmonids (Wilson and Poe, 1985; Pongmaneerat and Watanabe, 1993; Olli et al., 1994; Olli and Krogdahl, 1995; Carter et al., 1994; Storebakken et al., 1998; Carter and Hauler, 2000), soybean has been tested only once with eels (Degani, 1987). Degani (1987) found that the European eel (mean initial weight 2 g) did not utilise soybean meal efficiently compared to fish or chicken meal control diets. Results also indicated that higher replacement with soybean (up to 20%) was only possible with the higher inclusion of fish or chicken meals as well (Degani, 1987). In contrast, the present study indicated that 23% replacement of fish meal protein with solvent extracted soybean is possible since this diet produced similar growth and growth efficiencies to fish meal. Similar weight gain and high apparent digestibility coefficients obtained on

Table 7.1.

Formulation (g/kg) and chemical composition of the experimental diets

	Diets				
Ingredients(g/kg)	CON	SBM	LM	CGM	MM
Fish meal	454.0	320.0	320.0	320.0	320.0
DL-methionine	5.0	5.0	5.0	5.0	5.0
L-tryptophan	3.0	3.0	3.0	3.0	3.0
Blood meal	100.0	100.0	96.0	98.0	100.0
Soybean meal	0.0	195.0	0.0	0.0	0.0
Lupin meal	0.0	0.0	336.0	0.0	0.0
Corn gluten meal	0.0	0.0	0.0	148.0	0.0
Meat meal	0.0	0.0	0.0	0.0	135.0
Fish oil	100.0	113.0	98.0	105.0	103.0
Dextrin	107.0	100.0	90.0	100.0	100.0
Bentonite	162.8	95.8	0.0	152.8	165.8
∝-Cellulose	20.0	20.0	10.0	20.0	20.0
CMC	30.0	30.0	23.8	30.0	30.0
Minerals[1]	12.5	12.5	12.5	12.5	12.5
Vitamins[2]	5.0	5.0	5.0	5.0	5.0
Stay-C[3]	0.5	0.5	0.5	0.5	0.5
B.H.A	0.2	0.2	0.2	0.2	0.2
Chemical composition (g/kg DM)					
Dry matter	954.0±1.3	960.6±1.6	953.2±0.8	960.4±2.1	956.5±0.9
Crude protein	431.4±2.4	431.8±1.0	441.1±4.0	436.9±8.1	435.9±3.0
Crude fat	176.5±5.1	173.4±3.1	167.3±4.0	159.8±1.8	174.0±4.7
NFE[4]	135.9±13.1	200.7±46.0	278.7±0.6	158.3±37.8	132.3±4.8
Ash	210.3±11.7	154.8±43.5	66.0±7.8	204.5±30.1	214.4±4.2
GE(MJ/kg)	18.5±0.06	19.7±0.02	19.8±0.2	18.9±0.2	18.5±0.06
DCP	411.7	408.4	410.3	413.2	410.0
g DCP/MJ DE	22.25	20.73	20.72	21.86	22.16

[1]Mineral mixture (g/kg food): According to De la Higuera et al. (1989): CaH_2PO_4; 3.424, $CaCO_3$; 3.265, KH_2PO_4; 2.384; KCl; 0.24, NaCl; 1.442, $MnSO_4.H_2O$; 0.089, $FeSO_4.7H_2O$; 0.36, $MgSO_4$; 1.201, KI; 0.0046, $CuSO_4.5H_2O$; 0.012, $ZnSO_4.7H_2O$; 0.06, $CoSO_4$; 0.007, (Na_2MoO_4); 0.002, Na_2SeO_3; 0.005, $AlSO_4.18H_2O$; 0.004.

[2]Vitamin mixture (g/kg food): According to De la Higuera et al (1989): calcium pantothenate; 0.13,

thiamine; 0.044, riboflavin; 0.109, pyridoxine; 0.033, inositol; 0.874, biotin; 0.001, folic acid; 0.011, choline chloride; 2.623, nicotinic acid; 0.219, cyanocobalamin; 0.002, ascorbic acid; 0.874, retinol; 0.044, menadione; 0.022, ∝-tocopherol; 0.007, cholecalciferol; 0.009. Individual ingredients were supplied by Sigma-Aldrich Pty.Ltd. and ICN Biochemicals Pty.Ltd. Australia.

[3]Stay-C (L-Ascorbyl-2-polyphosphate) was supplied by Hoffman La Roche, Basil, Switzerland.

[4]Calculated as the remainder of crude protein+crude fat+ash (Carter and Hauler, 2000).

Table 7.2

The performance of the eel, *A.australis australis* fed diets containing different protein sources

	Diets					
Parameter	CON	SBM	LM	CGM	MM	*P*
Total initial weight (g)	44.51±0.31	44.30±0.43	44.33±0.80	44.93±0.81	44.62±0.60	ns
Total final weight (g)	57.90±1.01^{ab}	57.74±2.34^{ab}	50.13±1.78^{a}	58.91±8.32^{ab}	64.41±4.31^{b}	0.0349
Total weight gain (g)	13.41±1.10^{ab}	13.41±2.08^{ab}	5.90±0.41^{a}	13.90±7.76^{ab}	19.85±3.96^{b}	0.0245
Total feed consumption (g DM)	116.43±5.37	109.87±2.92	98.10±2.95	116.70±25.20	133.60±12.98	ns
SGR (%/d)	0.41±0.03^{ab}	0.43±0.05^{ab}	0.21±0.02^{a}	0.42±0.10^{ab}	0.58±0.10^{b}	0.0143
FER	0.12±0.01^{ab}	0.12±0.02^{ab}	0.06±0.004^{a}	0.12±0.04^{ab}	0.15±0.03^{b}	0.0160
PER (%)	30.41±0.51^{b}	30.00±5.43^{b}	14.67±1.01^{a}	27.73±9.08^{ab}	36.21±6.38^{b}	0.0090
PPV (%)	13.41±2.71^{b}	14.82±2.43^{b}	5.81±0.83^{a}	13.03±4.19^{ab}	16.93±2.54^{b}	0.0060
survival (%)	96.7±5.8	88.3±7.6	90	95.0±8.7	90	ns

Each value is the mean (± S.E.M) of triplicate tanks (n=3). Means in the same row with different superscripts are significantly different (Tukey-Kramer HSD, P<0.05).

Table 7.3

Chemical composition of the eel, *A.australis australis* fed diets containing different protein sources

	Diets					
Parameter	CON	SBM	LM	CGM	MM	*P*
Dry matter (%)	28.6±1.9	28.1±2.2	28.7±1.4	28.5±3.0	28.4±1.2	ns
Crude protein (% DM)	56.0±2.9	56.6±1.7	55.8±1.7	57.0±3.0	54.7±4.5	ns
Crude lipid (% DM)	27.8±4.3	28.0±4.5	25.9±3.9	27.8±5.3	27.6±5.5	ns

Each value is the mean (± S.E.M) of triplicate tanks (n=3).

Initial group (mean ± sd; n=6): 27.7±4.4 % dry matter; 58.2±2.6 % crude protein; 25.9±3.2 % crude lipid

Table 7.4.

Apparent digestibility coefficients (ADC, %) for dry matter (ADC_{DM}), crude protein (ADC_N) and energy (ADC_{kJ}) for the experimental diets consumed by the Australian short-finned eel

	Diets					*P*
Parameter	CON	SBM	LM	CGM	MM	
ADC_{DM}	62.1±4.6	69.4±3.5	72.9±4.5	72.3±1.2	61.4±4.1	ns
ADC_N	87.0±0.1[a]	90.7±0.1[b]	92.4±1.1[b]	88.1±0.3[a]	87.6±0.2[a]	0.0005
ADC_{kJ}	85.3±3.3[b]	85.1±0.5[b]	75.7±0.1[a]	85.1±0.2[b]	83.2±2.1[b]	0.0111

Each value is the mean (± S.E.M) of duplicate analysis of an equally pooled faecal samples collected for five days

Means with different letter in the same row are significantly different (Tukey-Kramer HSD multiple comparison)

Figure 7.1. Increase in total wet weight (g) by juvenile short-finned eel fed diets containing several plant and animal by-products over a 9-week period. Values are means ± S.E.M (n=3) for each treatment.

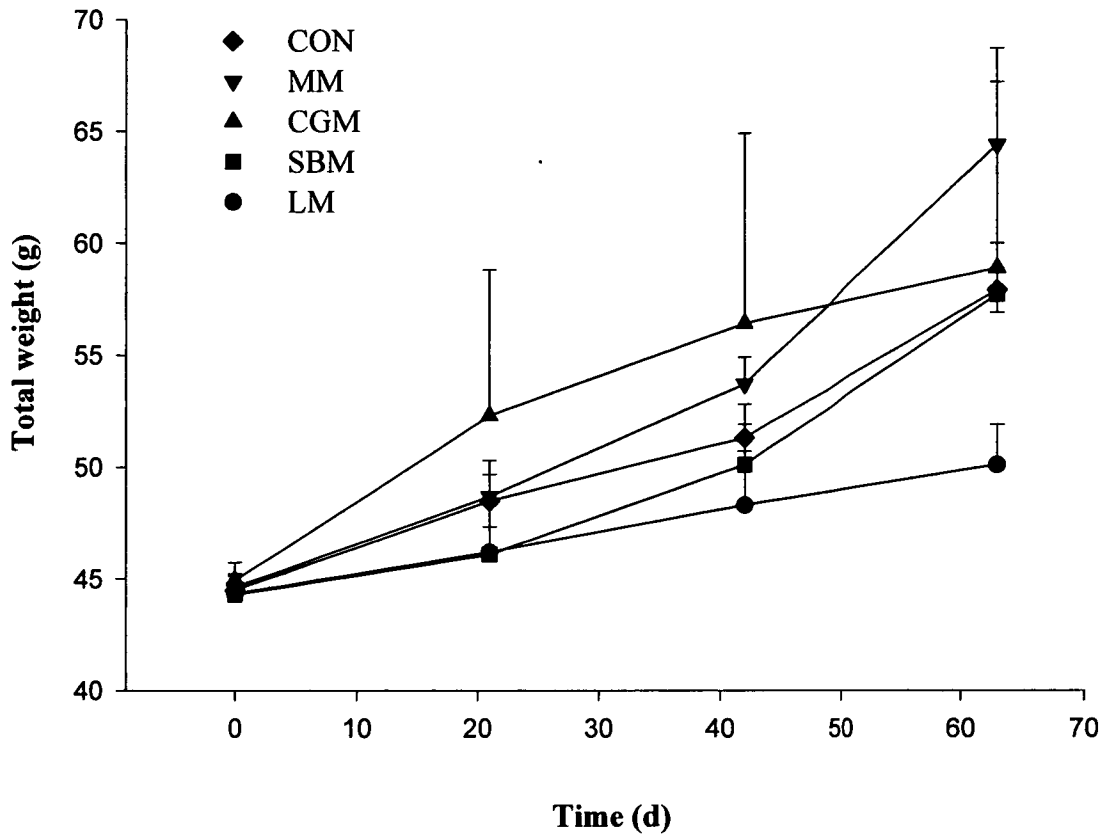

70
65
60
55
50
45
40
Total weight (g)
CON
MM
CGM
SBM
LM
0
10
20
30
40
50
60
70
Time (d)

Figure 7.2. Fluctuations of daily ammonia-nitrogen (NH_3-N) excretion by juvenile Australian short-finned eel fed diets containing several plant and animal by-product meals. Values are means ± S.E.M (n=3) for each treatment. [a] Represents initial mean ammonia-nitrogen values for each treatment.

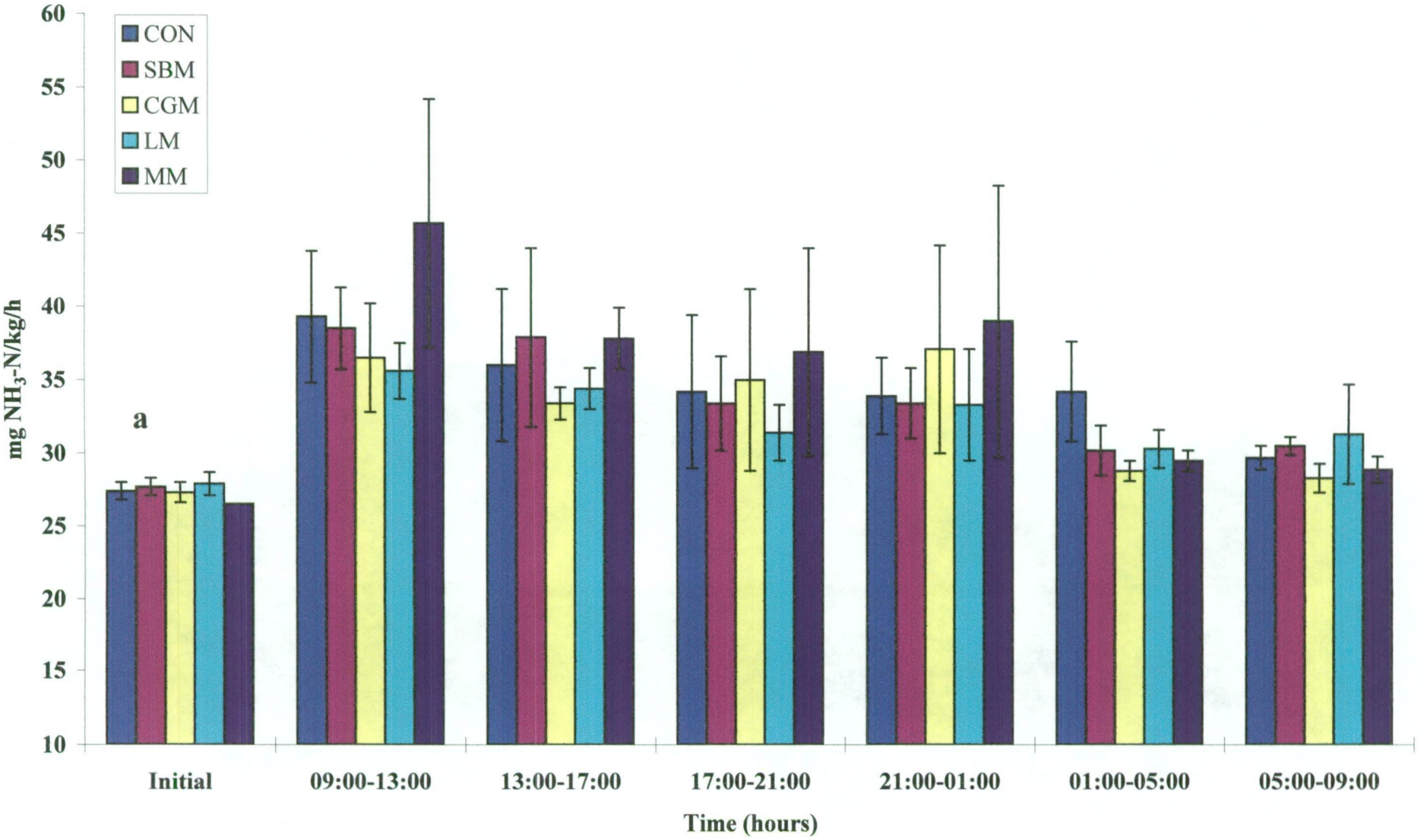
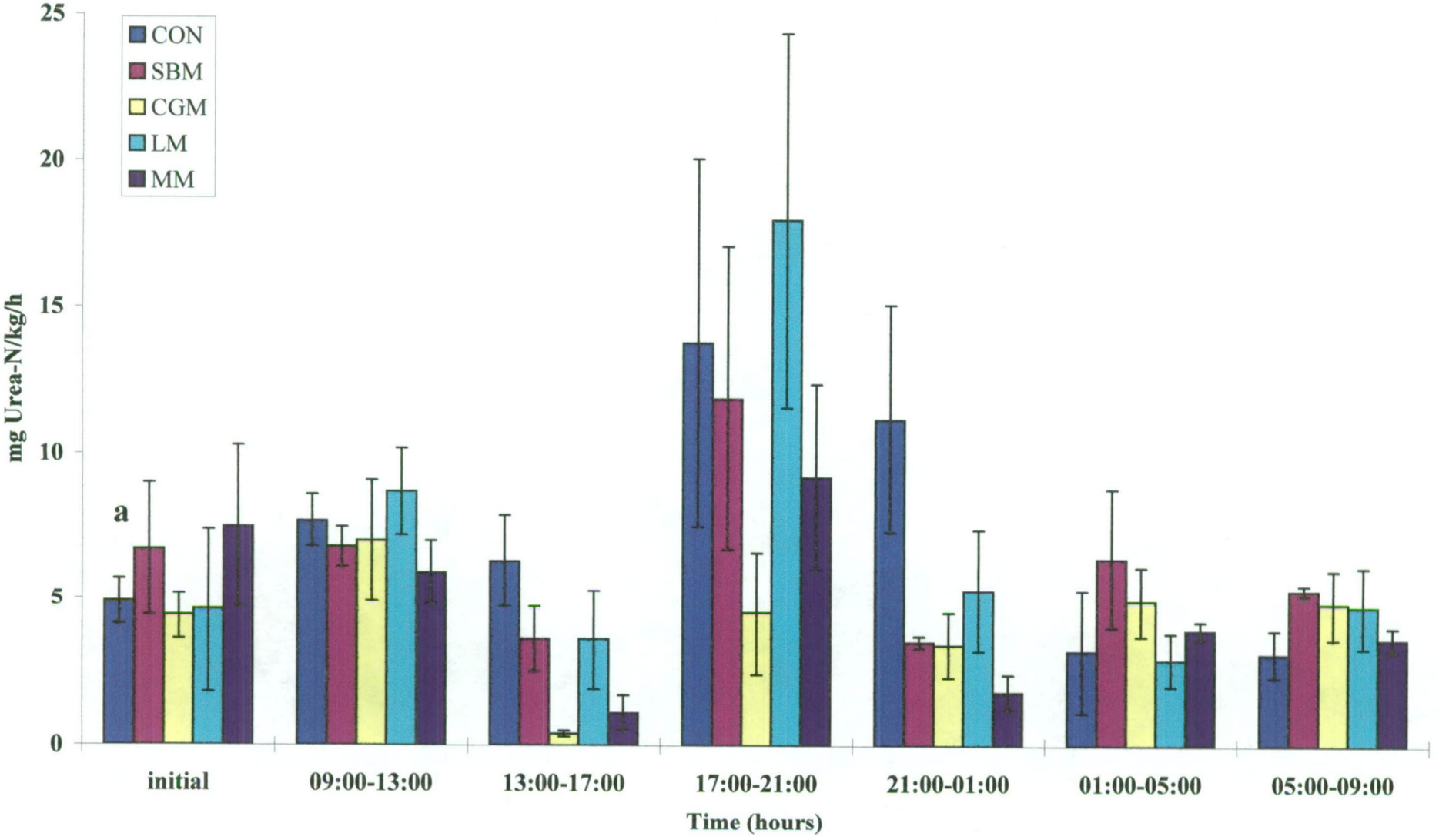

CON
SBM
CGM
LM
MM
60
55
50
45
40
35
30
25
20
15
10
mg NH3-N/kg/h
a
Initial
09:00-13:00
13:00-17:00
17:00-21:00
21:00-01:00
01:00-05:00
05:00-09:00
Time (hours)

Figure 7.3. Fluctuations of daily urea-nitrogen excretion by juvenile Australian short-finned eel fed diets containing several plant and animal by-product meals. Values are means ± S.E.M (n=3) for each treatment. [a] Represents initial mean urea-nitrogen values for each treatment.


CON
SBM
CGM
LM
MM
25
20
15
10
5
0
mg Urea-N/kg/h
a
initial
09:00-13:00
13:00-17:00
17:00-21:00
21:00-01:00
01:00-05:00
05:00-09:00
Time (hours)

SBM diet further suggested that decreased nutrient digestibility or palatability of diets containing soybean at this level of substitution were evident in the present study. The main limitation of substituting plant proteins for fish meal in large quantities is their low protein content, unbalanced amino acid profiles and several antinutritional factors such as complex carbohydrates and trypsin inhibitors (Mitchell et al., 1991; Gouveia et al., 1993; Refstie et al., 1998). Therefore, most of the research with salmonids and other fish species has concentrated on increasing the amount of plant proteins in fish diets and eliminating the harmful effects of these antinutritional factors with suitable processing techniques (Pongmaneerat and Watanabe, 1993; Carter et al., 1994; Olli et al., 1994; Refstie et al., 1998; Carter and Hauler, 2000). Compatible growth rate obtained on SBM diet to CON diet confirmes the efficiency of technological treatment involved in removing some of the antinutritional factors in soybean meal used in the present study (Médale et al., 1998). At 28% protein replacement only full fat soybean meal could maintain weight gain when compared to performances of Atlantic salmon obtained using dehulled and solvent extracted and solvent-extracted only soybean meals (Olli et al., 1994). Refstie et al. (1998) also demonstrated that 107 g Atlantic salmon raised in seawater had similar growth to fish meal control diet when fed a diet in which 40% of the fish meal protein was substituted with a soybean meal with reduced content of olligosaccharides, trypsin inhibitors, lectins and soya antigens. Refstie et al.(1998) also concluded that such high level of replacement was found possible due to significantly higher protein, fat and energy digestibility coefficients by this diet than toasted and extracted soybean meal.

To our knowledge the possibility of replacing fish meal protein with lupin meal (LM) has never been investigated in eel diets. Because of the low protein content in whole lupin seed meal, replacement (23% of fish meal protein on DM) was achieved only with a proportionally higher amount of lupin meal inclusion in LM diet (34% of the total ingredients used) (Table 7.1). Previous experiments with salmonid species (De la Higuera et al., 1988) showed that increased crude lupin seed meal content could lead to a progressively more difficult adaptation of fish to the organoleptic properties of such diets. Thus, lower feed consumption, although not significantly different than the other diets, in elvers in the present study may have been due to this and partly explain the growth depression on LM diet. Main factor affecting the

utilisation of the lupin seed meal in the present study appeared to be related to the chemical properties present in this meal. Australian sweet lupin strains have been reported to contain low alkaloids (<0.02%) and trypsin inhibitor (0.14 mg/g sample) (Petterson et al., 1997). The results obtained for protein digestibility in LM diet indicated that lupin meal was well digested by the elvers. Therefore, significantly lower growth and growth efficiencies were probably due to high amount of indigestible carbohydrate fraction of the lupin meal which resulted in significantly lower energy digestibility in elvers (Morales et al., 1994; Robaina et al., 1995).

Corn gluten is the major protein fraction from wet milling which separates corn into starch, germ, protein and fibre fractions (Park et al., 1997; Wu et al., 1999). Because of its high protein and low fibre content, corn gluten meal has been tested as a fish meal protein replacement in several fish species (Morales et al., 1994; Yamamoto et al., 1995; Robaina et al., 1997; Watanabe et al., 1997). However, its potential in eel diets is not known. In the present study elvers fed CGM diet showed similar growth and growth efficiencies to CON, SBM and MM diets. High nutrient digestibility values obtained on this diet further suggested that dietary energy efficiency is directly related to the amount of digestible energy in practical diets (Cho et al., 1982; García-Gallego et al., 1994; Morales et al., 1994). Therefore, carbohydrate content of CGM might have been highly digestible to elvers and contributed positively to the overall nitrogen retention efficiency in this diet. Similarly improved growth rates and feed utilisation were reported with rainbow trout when fed diets in which 23 or 40 % of fish meal or soybean protein concentrate were replaced with CGM (Morales et al., 1994; Watanabe et al., 1997).

Elvers fed meat meal (MM) demonstrated the highest growth and growth efficiencies in the present study. Increased feed consumption (Table 7.2) may partly explain the significantly higher growth rates. Higher consumption of MM diet may be related to the olfactory response of elvers to MM diet. In fact, eels were shown to have a stimulatory feeding activity when presented food extracts rich with arginine, alanine and glycine (Hashimoto, 1968; Yoshii et al., 1979; Mackie and Mitchell, 1983). Meat meal had the highest amount of arginine among the alternative protein sources (see Table 6.3) used in the present study. However, synergetic or additive interaction between amino acids from different protein sources or inclusion of free

essential amino acids (EAA) to diets is likely to create more potent stimulatory feeding activity in eels (Yoshii et al., 1979). Along with higher feed consumption, the lowest NFE (nitrogen free extract) content as a result of highly digestible ingredients in this diet appeared to have a positive effect on growth and growth efficiencies. However, the growth of the European eel has been shown to be affected negatively when the 50 % of the dietary fish meal protein was replaced with meat meal (García-Gallego et al., 1998) indicating an imbalance in the intake of essential and non-essential amino acids absorbed in quantities and patterns which are disproportianate to those required for optimum utilisation (D'Mello, 1994).

Dietary crude protein digestibilities in the present study were between 88 and 93 % and comparison with fish meal based CON diet indicated that both plant proteins and meat meal (MM) were well digested by juvenile short-finned eel. Since crude protein digestibility of the individual ingredients (see Table 6.4) were used to calculate a value for the complete diets in the present study, similarity between calculated and measured values confirmed the validity of the measurements (Carter and Hauler, 2000). High amounts of indigestible carbohydrates in lupin seed meal (LM) significantly lowered the energy digestibility of this diet by the short-finned eel. However, absence of a correlation between crude protein digestibility and either energy digestibility or ∝-cellulose content suggested that addition of indigestible carbohydrate did not influence protein digestibilities.

The use of non-fishmeal protein sources may necessitate the use of supplemental amino acids to restore the amino acid profile of the complete diets (Ketola, 1982; Davies and Morris, 1997). However, this supplementation is argued to be made to match an optimum pattern rather than simply to ensure that the minimum numerical requirement for each of the essential amino (EAA) acid is met (Cowey, 1995). In the present study, co-agulated and spray-dried blood meal was substituted at equal amounts along with DL-methionine and L-tryptophan (a separate approach) to bring the amino acid profile of diets in line with the requirements of the Japanese eel. Higher growth rates obtained on MM diet than all the other experimental diets indicated that the lowest sum of differences between the target and the actual amino acid profile might have been reached in this diet (Davies and Morris, 1997). Therefore, it would be correct to say that the short-finned eel was able to utilise the

amino acid supplementation made by a separate approach in the present study. However, the major issue in the present study was balancing the EAA profile of the diets rather than investigating the effects of supplementing or deleting individual essential amino acids on growth rates. Major drawbacks of a separate approach are the concerns over the digestibility and palatibility of a protein source which consist a major part of the diet (Cowey, 1995). Blood meal was found to be highly digestible by the similar size of short-finned eel (see Table 6.4). Thus, a balanced spectrum of available amino acids must have been released in the intestine of elvers fed the experimental diets. However, significantly lower growth and growth efficiencies and the absence of any changes in body nitrogen content in elvers fed LM diet suggests the interference of an anti-nutritional factor possibly the high amount of indigestible carbohydrate rather than the amino acid deficiency (Davies et al., 1997). García-Gallego et al. (1998) demonstrated that the European eel was able to utilise free amino acid supplementation efficiently since the growth rate of eels was twice higher when fed the diet in which 100 % of fish meal protein is replaced by sunflower meal (SFM) with lysine, methionine, histidine and threonine supplementation than the diet in which 50 % of fish meal protein is replaced with sunflower meal without EAA supplementation.

There is little known about the effects of different protein sources other than fish meal on nitrogenous excretion in teleosts (Médale et al., 1998). The magnitude of the nitrogenous excretion was lower than the previously observed excretion rates (Engin and Carter, 2001) in juvenile short-finned eel. This may be explained by both the achievement of optimum dietary digestible protein to digestible energy and the balanced of amino acids in the experimental diets thereby maximising nitrogen retention by minimising the deamination of amino acids (Cho and Kaushik, 1985; Kaushik and Cowey, 1990; Médale et al., 1995). Médale et al. (1998) was able to demonstrate a reduction in ammonia-nitrogen excretion by rainbow trout fed diets containing high amounts of SPC (soy protein concentrate) supplemented with DL-methionine. Regardless of the protein source in diets, ammonia-nitrogen excretion began to increase and peaked 4 h after the morning feed. This was in agreement with the findings of Robaina et al. (1995; 1997) with gilthead sea bream (*Sparus aurata*). However, the delay in ammonia-nitrogen excretion by gilthead bream fed plant proteins (soybean and lupin meal) (Robaina et al., 1995) was not experienced in the

present study indicating the time required for the digestion, absorption and metabolism of the plant proteins, at this replacement level, did not differ than that of animal-by products in the short-finned eel. The daily urea-nitrogen excretion rates were not affected by treatment apart from 4 h following the afternoon feed where excretion rates were two to three times higher in all the treatments except CGM. Similar observations were made with turbot (*Scopthalmus maximus* L.) fed a fish meal and greaves meal based diet (Dosdat et al., 1995) suggesting urea excretion could be the consequance of an active urea cycle that would explain the linkage between urea production and nitrogen intake in both turbot and short-finned eel. Since *de novo* synthesis of urea is energetically costly to teleosts requiring an input of at least 2.5 ATP per nitrogen excreted (Korsgaard et al., 1995; Walsh, 1998), the highest periodic urea excretion on LM could partly explain the depressed growth rate found with this diet.

In conclusion, partial replacement of fish meal protein with soybean meal, corn gluten meal and meat meal was demonstrated to be feasible in juvenile short-finned eel diets. However, raw lupin seed meal appeared to have a suppressive effect on feed consumption, growth and growth efficiencies of the short-finned eel most likely due to its high indigestible carbohydrate content. Supplemental essential amino acids to diets seemed to be utilised efficiently by the elvers. Measurements of digestibility and nitrogenous excretion were useful in extrapolating the growth rates obtained on treatments. More research is needed for the further improvement in the use of these alternative protein sources in diets for the Australian short-finned eel.

CHAPTER EIGHT

General Discussion

8.1. General discussion

Considerable interest exists in the Australian short-finned eel culture in Australia (Zeller and Beumer, 1996). However, there is little known about the nutritional requirements and feeding of this species. One of the most important aspects of a high density recirculating culture system, the dominant culture system for eel species around the world, is maintaining good water quality through improved nutritional quality of fish feeds. The present study addressed this issue by investigating various aspects of protein nutrition and metabolism of the short-finned eel.

8.2. Dietary carbohydrate and lipids as energy sources in eel diets

The Australian short-finned eel was shown to utilise dietary digestible lipid and carbohydrate effectively as energy sources in the present study. Although a limited range of dietary protein to energy ratios was investigated, it appeared that diets containing approximately 10% each carbohydrate and 10% lipid (close to a ratio of 0.9) at an intermediate protein level (35 to 45% DM) produced a greater protein sparing effect than other diets. The diet with the highest protein level with the lowest carbohydrate and lipid significantly reduced the growth rate suggesting the use of the majority of the protein for maintenance energy requirements (Steffens, 1996). However, the diet containing the highest non-protein energy sources with the lowest protein might have resulted in suboptimal protein intake and lower growth rate in the short-finned eel. The fact that the PER and PPV values were higher for the diets containing higher non-protein energy sources further suggests the efficient use of dietary protein in those diets. Similar findings were reported for other fish species (Page and Andrews, 1973; Davies, 1989; Hillestad and Johnsen, 1994; Lanari et al., 1994) and eels (Degani and Viola, 1987; García-Gallego et al., 1993; 1995; Hidalgo et al., 1993; Sanz et al., 1993).

Eels were previously shown that they had a comparatively better ability to utilise high levels of dietary digestible carbohydrates than the rainbow trout (García-Gallego et al., 1995). According to Sanz et al. (1993) the diets which promoted the highest growth were those where the gross energy was supplied by a higher percentage of carbohydrate rather than lipid. However, eel size appears to be the

determinant factor in the ability of utilising carbohydrates (Sanz et al., 1993). The bigger size eels (30-40 g average mean weight) were shown to react in a similar manner to omnivorous and herbivourus fish when fed high carbohydrate diets, referring to protein sparing effect of chorbohydrate and lipids on protein utilisation (De la Higuera, 1989; García-Gallego et al., 1993; Hidalgo et al., 1993). Above 40% dietary protein becomes unprofitable for omnivorous and herbivorous fish species. Investigations with smaller size eels, including the present study (2-6 g) suggested that eels of this size may have higher protein requirement, a reduced tendency towards fattening or is more sensitive to the negative effect of increasing dietary carbohydrate to protein ratio (Sanz et al., 1993; García-Gallego et al., 1995). Infact, the efficiency of transfer of nutrients from the diet to the fish is determined by endogenous and exogenous factors that operate simultaneously (Shearer, 1994). Further investigations towards understanding the optimum carbohydrate to fat ratio for a maximum growth in the short-finned eel diets may clarify the energy requirements and the storage of energetic reserves in the body.

8.3. The optimum dietary protein to energy requirement of the juvenile Australian short-finned eel

An experiment (Chapter 3) suggested that the optimum dietary protein to energy levels in the short-finned eel may approximately be 19 g CP/MJ GE, although it was not designed specifically to measure this. The findings prompted an investigation into the optimum protein to energy requirement in the juvenile short-finned eel in order to find the most efficient feed formulation through which growth rate is maximised. The dietary protein requirement is one of the most important aspects in the development of a nutritionally adequate feed. The results showed that the short-finned eel required 43.0 (±3.5) % dietary protein on a DM basis or 24.5 (±1.7) g DCP/MJ DE for a maximum growth when fed a fixed ration (5% BW/d). Optimum dietary protein to energy levels in several eel species are known (Table 5.5) and the present findings generally agreed with the previous requirement estimates of 40 to 47% of dietary protein (Nose and Arai, 1972; Degani et al., 1985; De la Higuera et al., 1989; Tibbetts et al., 2000). However, there seem to be some, although not larger than 6%, differences in estimated values within or between species attributable to different methodology: non-protein energy substitutes in diets; feeding regimes; fish

age classes and methods for the determination of dietary energy content (Tacon and Cowey, 1985).

The dietary optimum protein to energy ratios for fish species should be reported on a digestible basis (Tacon and Cowey, 1985). The requirement value is the protein concentration (fed as graded levels in isoenergetic diets) above which no significant increase in growth is found under the particular experimental conditions employed (Bowen, 1987; Shearer, 2000). The biggest challenge in nutrient requirement experiments is to decide on the appropriate experimental design and the analytical method to estimate the requirement levels (Shearer, 2000). Shearer (2000) stated that many of the previously published nutrient requirement studies with teleosts misinterpreted the results due to flaws in experimental designs such as inappropriate statistical methods to examine the dose-response relationship and poor model choice as a result of a failure to test for adequate model fit . These issues have been addressed in the present study by employing sufficiently low and high levels (judged by the previous findings for other eel species) of dietary digestible protein range and requirements were estimated with two different models (second order polynomial (quadratic) and 5-SKM (five-parameter saturation kinetics model)). The estimated values from both models were similar and the magnitude of differences (%) between them as DCP (%) and g.DCP/MJ DE were 94 and 95%, respectively (Table 5.4). This indicated the appropriate choice in selection of models for the evaluation of dose-response data obtained in the present study since a 100% value is used as a standard magnitude of difference in estimated values from two different models (Shearer, 2000).

Protein and amino acid requirements are a function of the metabolic demands of the organism and the efficiency with which diet can be utilised to meet the metabolic demand (Millward, 1998). Therefore, the diurnal nature of overall daily nitrogen balance (the assumption of fed state gains must balance fasting losses) might have been negatively affected in elvers fed diets which 47.5 and 55% crude protein with lower amounts of carbohydrates and lipids (Millward, 1998). In growing fish protein synthesis may account for as much as 42% of total energy expenditure being one of the most significant energy demanding process in the body (Houlihan et al., 1988). When dietary protein increases above the optimum ratio with non-protein energy, an

increase in the stimulation of protein synthesis occurs (Houlihan et al., 1995a). However, this protein is not retained as growth and therefore the retention of synthesised protein decreases. Thus, elvers fed diets with protein energy ratios that were either lower or higher than the diet producing the highest protein retention might have had higher anabolic stimulation efficiency and lower synthesis retention efficiency (Carter et al., 1995).

8.4. Ammonia- and urea-nitrogen excretion as influenced by dietary composition

The main end-product of protein metabolism in teleosts is ammonia and a significant proportion of nitrogenous waste is also excreted as urea (Jobling, 1981; Wood, 1993; Dosdat et al., 1995; Korsgaard et al., 1995). Nitrogen excretion in teleosts is affected by many factors such as species, rearing conditions, body weight, temperature and physiological status (Kaushik and Cowey, 1990). However, the level of ingested nitrogen appears to be the most important factor and influenced by the quantity or quality of the ingested diet (Dosdat et al., 1995). Therefore, quantification of ammonia- and urea-nitrogen excretion for fish species in relation to dietary variables is important in intensive fish culture since protein metabolism partly defines the success of a particular nutritional regimen (Dosdat et al., 1995; Gélineau et al., 1998).

In the present study, ammonia- and urea-nitrogen excretion by the juvenile short-finned eel were measured in relation to increasing dietary crude protein intake at two different energy levels (Chapter 4) and the combinations of alternative protein sources in balanced diets (Chapter 7). Increasing dietary protein in isoenergetic diets increased the ammonia-nitrogen excretion in the short-finned eel and it was in agreement with previous findings for eels (Gallagher and Matthews, 1987; Degani and Levanon, 1988). However, the amount of excretion was lower in diets with higher non-protein energy yielding substrates and further indicating the protein-sparing effects of these diets (Lied and Braaten, 1984; Jobling, 1994; Rodehutscord and Pfeffer, 1999). This was probably, as demonstrated for some other fish species (Walsh and Milligan, 1995; McGoogan and Gatlin, 1999), due to reduced glutaminase activity on higher dietary non-protein energy diets as ammonia is an

end-product of the metabolism of glutamine to glutamate. Eventhough the same feeding regime was used in all the experiments conducted in the present study, the pattern of excretion over time differed. The replacement of fish meal protein with plant and animal proteins did not result in two distinct peaks of ammonia-nitrogen excretion following the morning and afternoon feeds, as was demonstrated with fish meal based diets at constant energy levels (Chapter 3). Excretion rates were lower and began to increase 0-4 h after the morning feed in all the treatments and quickly returned to initial values. The implications for this may be the feeding elvers with optimum required dietary protein to energy levels, establishment of required amino acid pattern with intact protein sources and individual crystalline amino acid supplements or the similar time frame requirement for the digestion, absorption and metabolism of these protein sources to fish meal at 23% replacement level. Robaina et al. (1995) demonstrated a delayed ammonia-nitrogen excretion with gilthead sea bream fed a diet in which 40% of fish meal was replaced with soybean meal.

Urea-nitrogen excretion exhibited an increasing trend with increasing protein intake. This was in agreement with studies on the European eel (Masoni and Payan, 1974; Knights, 1985) and several flatfish species (Kikuchi, 1995; Carter et al., 1998; Verbeeten et al., 1999) and indicated that urea might be an important excretory metabolite in the Australian short-finned eel under certain conditions. The exceptional and unexpected daily pattern observed when elvers were fed balanced diets consisting combinations of protein sources (fish meal, blood meal, plant or animal by-product meals) suggested a strong specificity of short-finned eel urea excretion to protein sources (Dosdat et al., 1995). In the present study single high urea-nitrogen excretion peaked 12 h after the first feeding and 4 h after the beginning of the dark phase (Figure 7.3). The time lag for the apparition of peak urea excretion appears to be related to nitrogen intake. Although defining biochemical mechanism is not clear, it is generally assumed that urea released by ammoniatellic freshwater teleosts is produced mainly through uricolysis and corresponds to an endogenous rhythm (Dosdat et al., 1995; 1996; Korsgaard et al., 1995; Wright et al., 1995; Walsh, 1998). However, such specificity in urea excretion in some teleosts including in freshwater adapted eels could also be the result of the presence of active ornithine urea cycle (OUC), high affinity of mitocondrial arginase in the liver for dietary arginine since arginase is the last enzyme of the OUC that hydrolyses arginine into

ornithine and urea or the pulsatile release of urea stored in the urinary bladder (Mommsen and Walsh, 1991; Dosdat et al., 1995). These possible routes for urea excretion are yet to be confirmed in the Australian short-finned eel. Further investigations towards understanding the details of metabolic excretion in this species in relation to nutritional variables could make farming efforts more feasible by efficient feed formulations hence maximum protein utilisation and lower nutrient discharge in farm effluents.

8.5. Feed development studies for juvenile Australian short-finned eel

Rapidly expanding aquaculture production necessitates finding alternative protein sources to fish meal since fish meal is an expensive component of fish diets (Anderson et al., 1993; Hardy, 1996). The nutritional value of these protein sources is dependent on their bioavailability to fish species being fed (Kaushik, 1990; McGoogan and Reigh, 1996; Allan et al., 1998). Measurement of apparent digestibility coefficients for dry matter (ADC_{DM}), crude protein (ADC_{CP}) and energy (ADC_{kJ}) and availability of amino acids in protein sources have been used to evaluate the nutritional value of these products before being considered for fish meal replacement studies with fish species (Cho et al., 1982; Wilson and Poe, 1985; McGoogan and Reigh, 1996; Yamamoto et al., 1998).

Apart from chemical properties and availability of the nutrients, the methodology and protocole (time differences between feeding and the collection of faeces) used to collect digesta significantly affect the final digestibility values (Storebakken et al., 1998). Although Guelph settlement type faecal collectors (Cho et al., 1982) are widely used in digestibility studies with pelagic fish species, their use with eels is rather limited due to eel's bottom feeding habit and going through the openings to collectors (Tibbetts et al., 2000). However, a modification to a Guelph type settlement collector (Chapter 2) and its suitability in digestibility studies with juvenile short-finned eel was part of the investigation. The selection of Guelph settlement faecal collectors in the present study was due to imposibility of using other faeces collection techniques like stripping and dissecting the intestinal tract in juvenile fish (Schmitz et al.,1984). Digestibility coefficients reported for the same type of meals in different studies are currently hard to compare due to different

techniques used to measure digestibility, quality of the ingredients, dietary composition, fish size, ration level and the water temperature employed in the experiments (Wilson and Poe, 1985; Anderson et al., 1992; Watanabe et al., 1996; Yamamoto et al., 1997; da Silva and Oliva-Teles, 1998; Bureau et al.,1999). However, similar digestibility coefficients reported for eels (Schmitz et al., 1984) and other fish species like Australian silver perch (Allan et al., 1998) using almost similar or exactly the same type of collection technique to the present study may validate the modification.

The apparent crude protein digestibility coefficients ranged between 85 and 97% for all the plant and animal proteins tested in the present study. This suggested that the protein digestibility is not affected by dietary protein levels since plant proteins, specifically lupin meal and field pea meal, had significantly lower crude protein content than animal by-products (Watanabe and Pongmaneerat, 1993; De Silva et al., 2000). The apparent digestibility coefficients for dry matter and energy with plant proteins were significantly lower than that with animal by-products in the short-finned eel except when fed with corn gluten meal (CGM) suggesting the interference of high levels of indigestible carbohydrates or NFE in these meals (Refstie et al., 1998; Carter and Hauler, 2000). The higher dry matter and energy digestibility demonstrated for CGM may not be surprising since the European eel has been shown to utilise high levels of corn starch comparatively better than the rainbow trout (García-Gallego et al., 1993). Better dry matter and energy digestibilities found with soybean meal (SBM) and canola meal (CM) than LM and FPM diets may indicate the proper processing techniques applied to these products to alleviate the negative effects of anti-nutritional factors on digestibility since increased digestibility values were reported in labarotary animals and fish species fed processed soybean products (De la Higuera et al., 1988; Pongmeneerat and Watanabe, 1993; Olli and Krogdahl, 1994; Olli et al., 1994; Bureau et al., 1998; Hernández-Infante et al., 1998; Refstie et al., 1998).

All the animal by-products (meat meal, blood meal and poultry meal) were highly digestible by the short-finned eel (Table 6.4). Amino acid balance and the processing techniques during the production of animal by-products are considered to be the major factors on their digestibility with fish species (Nengas et al., 1996;

Bureau, 1999). However, high ash content in some of the meat and meat and bone meals was given as a likely reason for the lower apparent digestibility coefficients for crude protein, dry matter and energy (De Silva et al., 2000). In contrast to the findings of De silva et al. (2000), meat meal in the present study was found to be highly digestible indicating the substantial differences in the quality of the meat meals used, the fish size and the experimental conditions between the two studies.

An assumption is that digestibility coefficients for different ingredients are additive and can be used to formulate a complete diet (Cho et al., 1982; McNab, 1994; Allan et al., 1998). This assumption has been demonstrated using diets with range of ingredients and faeces collected either by dissection or settlement for rainbow trout, tilapia, channel catfish and ayu (Cho et al., 1982; Wilson and Poe, 1985; Watanabe et al., 1996). In the present study the effects of 23% fish meal protein replacement with soybean meal, corn gluten meal, lupin meal or meat meal on growth and growth efficiencies were investigated under the assumption of additivity of digestibility coefficients for different ingredients. Blood meal and small amounts of DL-methionine and L-tryptophan were used to bring the essential amino acid (EEA) profile of the diets in line with the requirements of the Japanese eel (Arai, 1991). The selection of blood meal was due to its high digestibility to the short-finned eel (Table 6.4) and high amounts of phenylalanine which appeared to be substantially deficient compared with other EAA' s in all the diets before blood meal was added. A separate approach to amino acid inclusion in the form of complete proteins is believed to be more effective than free amino acid supplementation in the utilisation of those amino acids (McNab, 1994; Davies and Morris, 1997).

Soybean meal, corn gluten meal and meat meal gave compatible growth rates to fish meal based control diet and suggested that 23% of fish meal protein in diets for the short-finned eel could be replaced with these alternative protein sources. The implication is that the higher level of digestible energy in relation to digestible protein in these diets might have increased the efficiency of dietary energy utilisation to promote energy retention and growth (Morales et al., 1994). However, lupin meal had a depressing effect on growth and growth efficiencies due partly to lower feed intake. Previous experiments with salmonid fish species showed that high levels of lupin meal in salmonid diets (over 30% of complete diets) could lead to a

progressively more difficult adaptation of fish to the organoleptic properties of such diets (De la Higuera et al., 1988). However, main factor for causing decreased growth appeared to be the high indigestible carbohydrate fraction of the lupin meal which resulted significantly lower energy digestibility in elvers (Morales et al., 1994; Robaina et al., 1995). The assumption of additivity of the digestibility coefficients for separate ingredients was valid for the short-finned eel since there was a similarity between calculated and measured values.

8.6. Differential growth of eels in captivity and implications for nutritional studies

As demonstrated for other eel species (Wickins, 1983; Seymour, 1984), the Australian short-finned eel showed substantial year to year variation in growth rates in the present study. Since eels have to be caught from the wild, they are the representative of a large genetic pool and can be classified as slow, moderate and fast growing fish (Degani and Gallagher, 1995). These growth differences were counteracted by using the smallest possible variability in individual initial fish weight. However, a similar experimental initial weight does not guarantee an equally similar age or step in the life cycle (García-Gallego et al., 1998). Therefore, certain degree of dispersion in feeding behaviour and growth rates is expected in artificial rearing of eel species (García-Gallego et al., 1998).

Overall data for food intake, weight increase and feeing efficiency in eels is reported to be lower compared with other fish species with a similar initial weight and in experiments with similar duration, irrespective of dietary treatments (Sanz et al., 1993; García-Gallego et al., 1998). The growth and growth efficiency results reported here were in agreement with findings with other eel species (De la Higuera et al., 1989; Hidalgo et al., 1993; Sanz et al., 1993; Degani and Gallagher, 1995).

8.7. Overall summary and future research possibilities

Following issues that warrants a further research effort in order to clarify the protein metabolism of the short-finned eel in captivity have been identified:

☛ The Australian short-finned eel has been shown to utilise dietary digestible carbohydrate and lipid effectively for the maximum utilisation of dietary nitrogen for growth. It appeared that a lipid to carbohydrate ratio of 0.8 to 0.9 with medium level crude protein content in diets is needed for maximum growth. However, research that specifically targets the maximum ratio of these non-protein energy yielding substrates over a range of constant dietary energy would be useful for detailed understanding of nitrogen metabolism. Investigations towards biochemical or hormonal mechanisms involving the carbohydrate metabolism in the short-finned eel is also needed for the clarification of dietary energy utilisation and storage of energetic reserves in the body.

☛ Dietary nitrogen intake influenced the ammonia-nitrogen excretion in a similar fashion to other teleostan fish in the short-finned eel. However, the urea-nitrogen excretion rate appeared to be not only higher than many other fish species but also more responsive to the nutritional variables tested in the present study. Similar dietary components and the nitrogen excretion rates in saltwater adapted short-finned eels should be investigated if retention of nitrogen is going to be maximised by doing so. Since urea is costly to organisms to excrete and previously European eel was shown to excrete significantly less urea-nitrogen in saltwater (Masoni and Payan, 1974; Knights, 1985), considerable benefit may be gained in terms of feed utilisation via increased productive protein value (PPV) in those feeds.

☛ The fact that dry matter and energy digestibilities of some of the plant proteins (soybean meal, canola meal and corn gluten meal) were significantly higher than raw seed meals (lupin meal and field pea meal) suggested a positive effect of processing on carbohydrate digestibility in those products. Therefore, research investigating suitable processing techniques and their effects on apparent digestibility coefficients and availability of amino acids with lupin and field pea meals may make their use in commercial eel diet formulations possible.

☛ 23% fish meal protein replacement with soybean meal, corn gluten meal and meat meal was found to be possible in diets for juvenile short-finned eel. Although the best growth was obtained with meat meal diet, its use in fish diets may not be

possible until sometime due to "Crutzfeld Jacob" disease in Europe where the majority of the cultured short-finned eels is exported to. Therefore in short term, research into improving level of fish meal protein substitution by corn gluten meal and soybean meal should be increased in diets for the short-finned eel..

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